PATENT COOPERATION TREATY

PLOUGMANN VINGTOFT & PARTNERS

3 AUG. 2001

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

DHM/KPI

WRITTEN OPINION

(PCT Rule 66)

To:

Plougmann,Vingtoft & Partners
P.O. Box 3007,
Sankt Annae Plads 11
DK-1021 KÖPENHAMN

Date of mailing *(day/month/year)*	01 -08- 2001
Applicant's or agent's file reference: 21497 pc1	REPLY DUE within 60 days from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK00/00406	17.07.2000	15.07.1999

International Patent Classification (IPC) or both national classification and IPC7
A61K 35/78, A61P 15/12

Applicant
Köpenhavns Universitet et al

1. This written opinion is the _____ (first, etc.) drawn by this International Preliminary Examining Authority.
2. This opinion contains indications relating to the following items:
   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application
3. The applicant is hereby invited to reply to this opinion.
   - **When?** See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).
   - **How?** By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.
   - **Also** For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4*bis*. For an informal communication with the examiner, see Rule 66.6.

   If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.
4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 15.11.2001

Name and mailing address of the IPEA/SE
Patent- och registreringsverket
Box 5055
S-102 42 STOCKHOLM
Facsimile No. 08-667 72 88
Telex: 17978 PATOREG-S

Authorized officer
Carolina Gómez Lagerlöf/BS
Telephone No. 08-782 25 00

WRITT[redacted]PINION

International application No.
PC[redacted]K00/00406

I. Basis of the opinion

1. With regard to the elements of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement) under article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*

WRITTEN OPINION

International application No.
PCT/DK00/00406

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 26-27, 30-52

because:

☒ the said international application, or the said claims Nos. 26-27, 30-52 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by therapy

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

WRITTEN OPINION

International application No.
PCT/DK00/00406

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	3-13,19-25,28-29	YES
	Claims	1-2,14-18	NO
Inventive step (IS)	Claims	28-29	YES
	Claims	1-25	NO
Industrial applicability (IA)	Claims	1-25,28-29	YES
	Claims		NO

2. Citations and explanations

During the search the following documents were found:
A Fortschr. Med. Med., Vol 114, No 5, 1996, pp 29-30
B Journal of women's health, Vol 7, No 5, 1998, 525-529
C EP 847755

Document A shows that extract from Cimcifuga (Remifemin) can be used in a stepped treatment plan in order to treat climacteric symptoms without stimulating the growth of the mammary carcinoma cells.

Document B shows that Cimcifuga racemosa is effective in the treatment of symptoms of menopause.

Document C discloses the use of an extract of Cimcifuga racemosa in the manufacture of a medicament for the treatment of estrogen induced tumours.

It is known that an extract of Cimcifuga racemosa can be used in the treatment of climacteric symptoms. It is also known to give the extract to patients as an estrogen replacement therapy so that the growth of the mammary carcinoma cells is not stimulated. It would be obvious to a person skilled in the art to give an extract of Cimcifuga racemosa to patients that suffer from or have a high risk of developing breast cancer, when it is known that the extract has the desired activity and does not stimulate mammary carcinoma cells.

Thus, claims 1-2 and 14-18 are not considered to fulfil the requirements of novelty and inventive step.
Claims 3-13 and 19-25 are considered to fulfil the requirement of novelty but not that of inventive step.
Claims 28-29 are considered to fulfil the requirements of novelty, inventive step and industrial applicability.

PLOUGMANN VINGTOFT & PARTNERS

Patent- och
Registreringsverket
Box 5055
S-102 42 Stockholm
Sweden

BY TELEFAX AND CONFIRMATION BY MAIL

COPENHAGEN OFFICE:
SANKT ANNÆ PLADS 11
POST OFFICE BOX 3007
DK - 1021 COPENHAGEN K
A/S REG. NO. 223795
TELEPHONE +45 33 63 93 00
TELEFAX +45 33 63 96 00
e-mail pv@pv.dk
www.pv.dk

Artist Bjørn Bjørnholt

AARHUS OFFICE:
NOBELPARKEN
JENS CHR. SKOUS VEJ 9
POST OFFICE BOX 49
DK - 8100 AARHUS C
TELEPHONE +45 87 39 18 00
TELEFAX +45 33 63 96 00
e-mail pv@pv.dk
www.pv.dk

Copenhagen, 1 October 2001

International Patent Application No. PCT/DK00/00406
Københavns Universitet
Cimicifuga-aktivstofprincipper
Our ref: 21497 PC 01

Dear Sirs,

Referring to the written opinion dated 1 August 2001, we hereby submit our response to the issues raised therein as well as a new set of claims.

New claims

In the new claims set previous claim 17 has been included in claim 1 which has made previous claim 17 and 25 superfluous, and these claims have thus been deleted. The subsequent claims have been renumbered accordingly. Furthermore has claim 1 been amended to clarify the scope of the claim by replacing "capable of inducing" with "which induces" so that the wording of the claim now is

> 1. Use of a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof, which induces a physiological estrogen-like effect without interacting with breast cancer cells, in particular without stimulating breast cancer cells, for the preparation of a medicament for the treatment of estrogen deficiency symptoms or diseases of a mammal suffering from or having a high risk of developing breast cancer.

Written Opinion

The Examiner cites three documents in the Written Opinion, the same three documents which were found during the International Search.

Document A was categorised as an X-document, whereas both B and C were categorised as A-documents.

It is therefore quite surprising that the Examiner now in her Written Opinion considers all the cited references to be relevant with respect to the assessment of novelty and inventive step of the present invention.

Document A

Document A is cited as showing "that extract from Cimicifuga (Remifemin) can be used in a stepped treatment plan in order to treat climacteric symptoms without stimulating the growth of the mammary carcinoma cells."

Nowhere in Document A can this latter statement regarding the lack of stimulatory effect on mammary carcinoma cells be found. Needless to say, no scientific indication of the this latter statement can be found in Document A.

Document A merely proposes a stepwise treatment plan for the treatment of climacteric symptoms, starting with placebo and placebo like substances, including phytotherapeutica, over non-hormone drugs and ending with hormone drugs as the ultimate treatment. Among the phytotherapeutics mentioned are extracts of Cimicifuga which are described as having estrogen like effect - a well known fact as also described in the description of the present application.

As the problem of treating climacteric symptoms with estrogen like substances is the risk of inducing proliferation of already recognised or possible estrogen receptor positive mammary carcinoma cells in the individual receiving the treatment, it does not necessarily make a phytotherapeutica the product of choice. From the scientific paper it seems as if it is accepted that no phytotherapeutica can have harmful effects, but this is a very dangerous assumption. This is very obvious from the literature, e.g. from Hilakivi-Clarke, L. Oncol Rep 1999 Sep-Oct;6(5):1089-95 wherein it is shown that the phytoestrogen genistein dose-dependently increased the incidence of DMBA-induced mammary tumours (abstract enclosed).

Without the scientific proof that phytoestrogen of Cimicifuga does not stimulate mammary carcinoma cells, it is highly irresponsible to propose the use of a compound having estrogen like effect for the treatment of patients with climacteric symptoms who are suffering from or having a high risk of developing breast cancer.

The new and highly surprising discovery which is the basis of the present application is that extracts of Cimicifuga have effects on the uterus and on mammary carcinoma cells both present in the same individual which are highly differentiated. The surprising and documented finding of the present application is that the phytoestrogen of Cimicifuga will stimulate the uterus but not the mammary carcinoma cells.

Document A does not disclose this very important information, simply because it was not known to the authors of the document.

It is furthermore obvious when going through table 3 of Document A that the potential effect of extracts of Cimicifuga was not realised. Extracts of Cimicifuga are not mentioned at all in this table, not even for the treatment of estrogen receptor negative mammary carcinoma.

The Examiner's conclusion that extracts of Cimicifuga can be used in the treatment of climacteric symptoms without stimulating mammary carcinoma cells cannot be drawn from Document A.

Document B

Document B is merely to be considered as an A-document such as it has also been categorised in the International Search. No other information than the well known estrogenic effect of extracts of Cimicifuga is disclosed in this document. The only conclusions are that the extracts of Cimicifuga are non-toxic, have no serious side effects (whatever that means) and have no mutagenic properties. There is no evidence or documentation that extracts of Cimicifuga do not stimulate mammary carcinomas.

Document C

Document C is also to be considered as an A-document which merely describes the background of the art. What is described in this document is a pharmaceutical composition for the treatment of cancer wherein an extract of Cimicifuga is one of the active constituents. The observation leading to the use of extracts of Cimicifuga in this pharmaceutical composition is that an extract of Cimicifuga not only augments the proliferation of estrogen-dependent tumour cells, but instead, in combination with an anti-estrogenic compound, markedly augments the proliferation-inhibiting effect of this latter compound. The differential effect of extracts of Cimicifuga as described in the present application cannot be found in this document.

Furthermore, an important factor of this document should be noted: All the results are obtained *in vitro*, i.e. it is clearly not possible from this document to evaluate the differential effect on both the uterus and the estrogen-dependent tumour cells in one individual.

A Japanese research group (Wei Sheng Yan Jiu 2001 Mar;30(2):77-80) has recently shown that extracts of Cimicifuga have estrogen effect on uterus weight *in vivo*, and at the same time shown that extracts of Cimicifuga stimulate the *in vitro* proliferation of the mammary carcinoma cell line MCF-7, the same cell line as used in Document C, i.e. it is impossible to conclude the *in vivo* effect as shown in the present application from *in vitro* experiments.

In view of what is stated above, the applicant finds that the subject-matter of the new claims fulfils the requirements of Art. 5, 6 and 33(2) PCT.

In case the Examiner does not agree that the new claims are properly based on the documents originally filed, and that the invention defined in the new claims is

novel and involves an inventive step, a personal interview with the Examiner pursuant to Rule 66.6 PCT is requested prior to the issuance of a preliminary examination report.

Yours sincerely,

Plougmann, Vingtoft & Partners

Peter Horn Møller

Abstracts of scientific papers
New claim set (incl. copy with amendments shown)

Claims

1. Use of a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof, which induces a physiological estrogen-like effect without interacting with breast cancer cells, in particular without stimulating breast cancer cells, for the preparation of a medicament for the treatment of estrogen deficiency symptoms or diseases of a mammal suffering from or having a high risk of developing breast cancer.

2. Use according to claim 1, wherein the composition which induces a physiological estrogen-like effect without stimulating breast cancer cells.

3. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is uterine growth as determined by an increase in uterine weight compared to controls after administration of the composition to ovariectomized female athymic nude mice.

4. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is uterine growth as determined by an increase in mean uterine weight compared to controls of at least 0.10 g after administration of the composition to ovariectomized NMRI female athymic nude mice for 8 days.

5. Use according to any of claims 3 or 4, wherein the increase in uterine weight obtained by administration of a dose comparable to a normal dose for the mammal to be treated of the composition corresponds to a weight increase obtainable in the same test animal by estradiol treatment.

6. Use according to any of claims 3 or 4, wherein the increase in uterine weight obtained by administration of a dose comparable to a normal dose for the mammal to be treated of the composition corresponds to a substantially maximum weight increase obtainable in the same test animal by estrogen treatment.

7. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is a change in gonadotropins (FSH and/or LH) as determined by available validated radioimmuno assay techniques.

8. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is a change in cytology of the vaginal cells as determined by cytological counts.

9. Use according to any of the preceding claims, wherein the composition do not interact, in particular stimulate, cancer cells that are estrogen receptor-negative.

10. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the composition compared to a control on growth of the estrogen and progesterone receptor negative MDA-MB-231 (ATCC HTB-26) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

11. Use according to any of the preceding claims, wherein the composition do not interact, in particular stimulate, cancer cells that are estrogen receptor-positive.

12. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the composition compared to a control on growth of the estrogen dependent and estrogen receptor-positive MCF-7 (ATCC HTB-22) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

13. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the composition when given in combination with estradiol compared to a control on growth of the estrogen dependent and estrogen receptor-positive MCF-7 (ATCC HTB-22) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

14. Use according to any of the preceding claims for the treatment of estrogen deficiency symptoms or diseases of humans having breast cancer, having a high risk of recurrent breast cancer, or having a risk (such as high risk) of developing breast cancer.

15. Use according to any of the preceding claims, wherein the estrogen deficiency-conditioned symptom or disease is selected from the group consisting of menopausal symptoms; dermatological disorders such as ageing of the skin, wrinkles, dry skin and other estrogen deficiency related dermatological disorders; dryness of mucous membranes (e.g. vaginal and intestine); brain related disease such as Alzheimer's including other types of dementia; bone and joint related diseases such as osteoporosis, osteochondrosis, osteoarthritis, rheumatoid arthritis, healing of bone fractures, and reduction in skeletal fractures; vaginal estrogen deficiency such as vaginal dryness and dyspareuni; coronary heart diseases such as arteriosclerosis; and disease such as hyperlipidaemia and hypercholesterolaemia.

16. Use according to any of the preceding claims, wherein the estrogen deficiency-conditions symptoms are menopausal symptoms.

17. Use according to any of the preceding claims, wherein the composition is or contains *Cimicifuga Racemosa* extract.

18. Use according to any of the preceding claims, wherein the composition is a composition comprising *Cimicifuga Racemosa* plant parts.

19. Use according to any of the preceding claims, wherein the composition is a composition comprising SPP-001.

20. Use according to any of the preceding claims, wherein the composition is a composition containing one or more chemical compounds contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

21. Use according to any of the preceding claims, wherein the composition is combined with a drug which has a selective estrogen receptor modulating (SERM) actitivty.

22. A container comprising a composition according to any of the preceding claims with a pharmaceutically carrier and comprising an indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening estrogen dependent cancer.

23. A container comprising a composition which induces a physiological estrogen-like effect without stimulating breast cancer cells with a pharmaceutically carrier and comprising an indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening estrogen dependent cancer.

24. A method for relieving symptoms caused by estrogen deficiency in a mammal suffering from or having a high risk of developing an estrogen dependent tumour comprising administering to the mammal a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof, which induces a physiological estrogen-like effect without stimulating breast cancer cells.

25. A method according to the previous claim, wherein the mammal is a human.

26. A method for screening for substances or compositions which can be used according to claim 1 or 2, comprising subjecting test substances or compositions to

1) testing for possible estrogen-like effect in normal tissue by measuring increase in uterine weight, changes in gonadotropins, changes in vaginal cytology and/or post-menopausal symptoms in an adult female mammal and
2) testing for possible estrogenic effect in breast cancer, and selecting, as candidates for tissue-selective estrogenic substances or compositions useful in the method according to claim 1 or 2, substances or compositions which,
   a) are capable of inducing physiological estrogenic effects in female mammals, and at the same time
   b) have no effect on the growth of estrogen receptor-negative cancer cells and no effect on estrogen receptor-positive cancer cells in the doses in which they induce physiological estrogen effects.

27. A method according to claim 26, wherein the capability of the substance or composition of inducing physiological estrogen effects e.g. uterine growth female mammals is tested by testing the capability of the substance or composition of effecting uterine weight increase in ovariectomized female NMRI athymic nude mice, the lack of effect of the substance or composition on the growth of estrogen receptor-negative cancer cells is assessed as the lack of capability of the substance or composition of supporting growth of MDA-MB231 xenografts in female NMRI athymic nude mice, and the lack of effect of the substance or composition on the growth of estrogen receptor-positive cancer cells is as-

sessed as the lack of capability of the substance or composition of supporting growth of MCF-7 (ATCC (HTB-22) xenografts in female NMRI athymic nude mice.

28. A method for relieving or curing symptoms or diseases which are caused by estrogen deficiency, or which can be relieved or cured by administration of steroidal estrogen, in a mammal who suffers from breast cancer, or has a risk of recurrent breast cancer, or has a high risk of developing breast cancer,
the method comprising administering, to the mammal, a composition
which has an estrogen-like effect, as evidenced by a capability of the composition of inducing physiological estrogenic effects in adult mammal, and
which is free from interaction with breast cancer cells, in particular free from a stimulating effect on breast cancer,
thereby treating estrogen deficiency symptoms or diseases without introducing a risk of provoking the development of clinically evident breast cancer and/or stimulating growth of existing breast cancer cells in the mammal.

29. A method according to claim 28, wherein the mammal is female mammal.

30. A method according to claim 29, wherein the female mammal is a woman.

31. A method according to any of claims 29-30, wherein the estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing an increase in uterine weight in adult ovariectomized NMRI female athymic nude mice.

32. A method according to claim 31, wherein the increase in uterine weight following a dose comparable to a normal dose for the mammal to be treated corresponds to a weight increase seen in the same test animal following estradiol treatment.

33. A method according to claim 32, wherein the increase in uterine weight following a dose comparable to a normal dose for the mammal to be treated corresponds to a substantially maximum weight increase obtainable in the same test animal by estrogen treatment.

34. A method according to any of claims 28-33, wherein the estrogen-like

effect possessed by the composition manifests itself in the composition being capable of inducing a lowering in FSH and LH in females.

35. A method according to any of claims 30-34, wherein the estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing an estrogen like change in vaginal cytology in females.

36. A method according to any of claims 28-35, wherein the composition is one which has no effect on the growth of estrogen receptor-negative cancer cells.

37. A method according to claim 36, wherein the composition is one which has no effect on the growth of xenografts of the estrogen and progesteron receptor-negative MDA-MB-231 (ATCC HTB-26) human breast cancer cell line in nude mice.

38. A method according to any of claims 28-37, wherein the composition is one which is free from any effect on breast cancer cells even where the breast cancer cells are documented as being estrogen receptor-positive.

39. A method according to any of claims 28-38, wherein the composition is one which has substantially no agonizing and substantially no antagonizing effect on the effect of estrogen such as estradiol on breast cancer cells, even where the breast cancer cells are documented as being estrogen receptor-positive.

40. A method according to claim 39, wherein the composition is one which substantially does not bind to estrogen receptors of cancer cells.

41. A method according to any of claims 38-40, wherein the composition is one which has no effect on xenografts of the estrogen receptor-positive and estrogen dependent MCF-7 (ATCC HTB-22) human breast cancer cell line in nude mice, as evidenced by the composition having no growth supportive effect and no growth inhibitory effect on the xenografts whether given alone or in combination with estradiol.

42. A method according to claim 41, wherein the composition is one which has no effect on xenografts of the estrogen receptor-positive and estrogen dependent MCF-7 (ATCC HTB-22) human breast cancer cell line in nude mice, as evidenced by the composition

having no growth supportive effect and no growth inhibitory effect on the xenografts whether given alone or in combination with estradiol, even where the composition is administered in a dose which is 10 or even 100 times higher than a dose giving, in the same strain of nude mice, a maximum uterus weight increase.

43. A method according to any of claims 28-42, wherein the estrogen deficiency-conditioned symptom or disease is selected from the group consisting of menopausal symptoms, dermatological disorders such as ageing of the skin, dryness of mucous membranes (e.g. vaginal and intestine), brain related disease such as Alzheimer's including other types of dementia, bone and joint related disease such as osteoporosis, osteochondrosis, osteoarthritis, rheumatoid arthritis, healing of bone fractures, and reduce in skeletal fractures and disease such as hyperlipidaemia, hypercholesterolaemia, arteriosclerosis.

44. A method according to claim 43, wherein the estrogen deficiency-conditions symptoms are menopausal symptoms.

45. A method according to any of claims 28-44, wherein the composition is a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

46. A method according to claim 45, wherein the composition is or contains *Cimicifuga Racemosa* extract.

47. A method according to claim 45, wherein the composition is a composition comprising *Cimicifuga Racemosa* plant parts.

48. A method according to claim 45, wherein the composition is a composition comprising SPP-001.

49. A method according to claim 46, wherein the composition is a composition containing one or more chemical compounds contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

50. A method according to claim 28, wherein the composition is combined with a drug which has a selective estrogen receptor modulating (SERM) activity.

Copy for the Elected Office (EO/US) PCT/DK00/00406

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PLOUGMANN & VINGTOFT A/S
Sankt Annæ Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
DANEMARK

Date of mailing (day/month/year)
29 January 2002 (29.01.02)

Applicant's or agent's file reference
21497 PC 1

IMPORTANT NOTIFICATION

International application No.
PCT/DK00/00406

International filing date (day/month/year)
17 July 2000 (17.07.00)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
PLOUGMANN, VINGTOFT & PARTNERS A/S
Sankt Annæ Plads 11
DK-1250 København K
Denmark

State of Nationality:
State of Residence:
Telephone No.: +45 33 63 93 00
Facsimile No.: +45 33 63 96 00
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
PLOUGMANN & VINGTOFT A/S
Sankt Annæ Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
Denmark

State of Nationality:
State of Residence:
Telephone No.: +45 33 63 93 00
Facsimile No.: +45 33 63 96 00
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Jaime LEITAO

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 004624672

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
21497 pc1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK00/00406	17.07.2000	15.07.1999
International Patent Classification (IPC) or national classification and IPC7		
A 61 K 35/78, A 61 P 15/12		
Applicant		
Köpenhavns Universitet et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13.02.2001	23.11.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Carolina Gómez Lagerlöf/EÖ Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/DK00/00406

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-41 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statement) under article 19
pages _____ , filed with the demand
pages 1-7 , filed with the letter of 01.10.2001

☒ the drawings:
pages 1-4 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/DK00/00406

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 24-50

because:

☒ the said international application, or the said claims Nos. 24-50
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/DK00/00406

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-23	YES
	Claims		NO
Inventive step (IS)	Claims	1-23	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-23	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The examination is based on the claims filed with the letter of 01-10 2001.

During the search the following documents were found:
A Fortschr. Med. Med., Vol 114, No 5, 1996, pp 29-30
B Journal of women's health, Vol 7, No 5, 1998, 525-529
C EP 847755

Document A shows that extract from Cimcifuga (Remifemin) can be used in a stepped treatment plan in order to treat climacteric symptoms without stimulating the growth of the mammary carcinoma cells.

Document B shows that Cimcifuga racemosa is effective in the treatment of symptoms of menopause.

Document C discloses the use of an extract of Cimcifuga racemosa in combination with a substance with antiestrogen activity in the manufacture of a medicament for the treatment of estrogen induced tumours.

It is known that an extract of Cimcifuga racemosa can be used in the treatment of climacteric symptoms. It is also known to give a composition including the extract to patients as an estrogen replacement therapy so that the growth of the mammary carcinoma cells is not stimulated. However, it is not known in the prior art that the extract has these two effects in the same individual.
The documents show the general state of the art.

Thus, claims 1-23 are considered to fulfil the requirements of novelty, inventive step and industrial applicability.

Claims

1. Use of a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof, which induces a physiological estrogen-like effect without interacting with breast cancer cells, in particular without stimulating breast cancer cells, for the preparation of a medicament for the treatment of estrogen deficiency symptoms or diseases of a mammal suffering from or having a high risk of developing breast cancer.

2. Use according to claim 1, wherein the composition which induces a physiological estrogen-like effect without stimulating breast cancer cells.

3. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is uterine growth as determined by an increase in uterine weight compared to controls after administration of the composition to ovariectomized female athymic nude mice.

4. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is uterine growth as determined by an increase in mean uterine weight compared to controls of at least 0.10 g after administration of the composition to ovariectomized NMRI female athymic nude mice for 8 days.

5. Use according to any of claims 3 or 4, wherein the increase in uterine weight obtained by administration of a dose comparable to a normal dose for the mammal to be treated of the composition corresponds to a weight increase obtainable in the same test animal by estradiol treatment.

6. Use according to any of claims 3 or 4, wherein the increase in uterine weight obtained by administration of a dose comparable to a normal dose for the mammal to be treated of the composition corresponds to a substantially maximum weight increase obtainable in the same test animal by estrogen treatment.

7. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is a change in gonadotropins (FSH and/or LH) as determined by available validated radioimmuno assay techniques.

8. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is a change in cytology of the vaginal cells as determined by cytological counts.

9. Use according to any of the preceding claims, wherein the composition do not interact, in particular stimulate, cancer cells that are estrogen receptor-negative.

10. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the composition compared to a control on growth of the estrogen and progesterone receptor negative MDA-MB-231 (ATCC HTB-26) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

11. Use according to any of the preceding claims, wherein the composition do not interact, in particular stimulate, cancer cells that are estrogen receptor-positive.

12. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the composition compared to a control on growth of the estrogen dependent and estrogen receptor-positive MCF-7 (ATCC HTB-22) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

13. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the composition when given in combination with estradiol compared to a control on growth of the estrogen dependent and estrogen receptor-positive MCF-7 (ATCC HTB-22) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

14. Use according to any of the preceding claims for the treatment of estrogen deficiency symptoms or diseases of humans having breast cancer, having a high risk of recurrent breast cancer, or having a risk (such as high risk) of developing breast cancer.

15. Use according to any of the preceding claims, wherein the estrogen deficiency-conditioned symptom or disease is selected from the group consisting of menopausal symptoms; dermatological disorders such as ageing of the skin, wrinkles, dry skin and other estrogen deficiency related dermatological disorders; dryness of mucous membranes (e.g. vaginal and intestine); brain related disease such as Alzheimer's including other types of dementia; bone and joint related diseases such as osteoporosis, osteochondrosis, osteoarthritis, rheumatoid arthritis, healing of bone fractures, and reduction in skeletal fractures; vaginal estrogen deficiency such as vaginal dryness and dyspareuni; coronary heart diseases such as arteriosclerosis; and disease such as hyperlipidaemia and hypercholesterolaemia.

16. Use according to any of the preceding claims, wherein the estrogen deficiency-conditions symptoms are menopausal symptoms.

17. Use according to any of the preceding claims, wherein the composition is or contains *Cimicifuga Racemosa* extract.

18. Use according to any of the preceding claims, wherein the composition is a composition comprising *Cimicifuga Racemosa* plant parts.

19. Use according to any of the preceding claims, wherein the composition is a composition comprising SPP-001.

20. Use according to any of the preceding claims, wherein the composition is a composition containing one or more chemical compounds contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

21. Use according to any of the preceding claims, wherein the composition is combined with a drug which has a selective estrogen receptor modulating (SERM) actitivty.

22. A container comprising a composition according to any of the preceding claims with a pharmaceutically carrier and comprising an indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening estrogen dependent cancer.

23. A container comprising a composition which induces a physiological estrogen-like effect without stimulating breast cancer cells with a pharmaceutically carrier and comprising an indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening estrogen dependent cancer.

24. A method for relieving symptoms caused by estrogen deficiency in a mammal suffering from or having a high risk of developing an estrogen dependent tumour comprising administering to the mammal a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof, which induces a physiological estrogen-like effect without stimulating breast cancer cells.

25. A method according to the previous claim, wherein the mammal is a human.

26. A method for screening for substances or compositions which can be used according to claim 1 or 2, comprising subjecting test substances or compositions to

1) testing for possible estrogen-like effect in normal tissue by measuring increase in uterine weight, changes in gonadotropins, changes in vaginal cytology and/or post-menopausal symptoms in an adult female mammal and
2) testing for possible estrogenic effect in breast cancer, and selecting, as candidates for tissue-selective estrogenic substances or compositions useful in the method according to claim 1 or 2, substances or compositions which,
   a) are capable of inducing physiological estrogenic effects in female mammals, and at the same time
   b) have no effect on the growth of estrogen receptor-negative cancer cells and no effect on estrogen receptor-positive cancer cells in the doses in which they induce physiological estrogen effects.

27. A method according to claim 26, wherein the capability of the substance or composition of inducing physiological estrogen effects e.g. uterine growth female mammals is tested by testing the capability of the substance or composition of effecting uterine weight increase in ovariectomized female NMRI athymic nude mice, the lack of effect of the substance or composition on the growth of estrogen receptor-negative cancer cells is assessed as the lack of capability of the substance or composition of supporting growth of MDA-MB231 xenografts in female NMRI athymic nude mice, and the lack of effect of the substance or composition on the growth of estrogen receptor-positive cancer cells is as-

sessed as the lack of capability of the substance or composition of supporting growth of MCF-7 (ATCC (HTB-22) xenografts in female NMRI athymic nude mice.

28. A method for relieving or curing symptoms or diseases which are caused by estrogen deficiency, or which can be relieved or cured by administration of steroidal estrogen, in a mammal who suffers from breast cancer, or has a risk of recurrent breast cancer, or has a high risk of developing breast cancer,
the method comprising administering, to the mammal, a composition
which has an estrogen-like effect, as evidenced by a capability of the composition of inducing physiological estrogenic effects in adult mammal, and
which is free from interaction with breast cancer cells, in particular free from a stimulating effect on breast cancer,
thereby treating estrogen deficiency symptoms or diseases without introducing a risk of provoking the development of clinically evident breast cancer and/or stimulating growth of existing breast cancer cells in the mammal.

29. A method according to claim 28, wherein the mammal is female mammal.

30. A method according to claim 29, wherein the female mammal is a woman.

31. A method according to any of claims 29-30, wherein the estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing an increase in uterine weight in adult ovariectomized NMRI female athymic nude mice.

32. A method according to claim 31, wherein the increase in uterine weight following a dose comparable to a normal dose for the mammal to be treated corresponds to a weight increase seen in the same test animal following estradiol treatment.

33. A method according to claim 32, wherein the increase in uterine weight following a dose comparable to a normal dose for the mammal to be treated corresponds to a substantially maximum weight increase obtainable in the same test animal by estrogen treatment.

34. A method according to any of claims 28-33, wherein the estrogen-like

effect possessed by the composition manifests itself in the composition being capable of inducing a lowering in FSH and LH in females.

35. A method according to any of claims 30-34, wherein the estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing an estrogen like change in vaginal cytology in females.

36. A method according to any of claims 28-35, wherein the composition is one which has no effect on the growth of estrogen receptor-negative cancer cells.

37. A method according to claim 36, wherein the composition is one which has no effect on the growth of xenografts of the estrogen and progesteron receptor-negative MDA-MB-231 (ATCC HTB-26) human breast cancer cell line in nude mice.

38. A method according to any of claims 28-37, wherein the composition is one which is free from any effect on breast cancer cells even where the breast cancer cells are documented as being estrogen receptor-positive.

39. A method according to any of claims 28-38, wherein the composition is one which has substantially no agonizing and substantially no antagonizing effect on the effect of estrogen such as estradiol on breast cancer cells, even where the breast cancer cells are documented as being estrogen receptor-positive.

40. A method according to claim 39, wherein the composition is one which substantially does not bind to estrogen receptors of cancer cells.

41. A method according to any of claims 38-40, wherein the composition is one which has no effect on xenografts of the estrogen receptor-positive and estrogen dependent MCF-7 (ATCC HTB-22) human breast cancer cell line in nude mice, as evidenced by the composition having no growth supportive effect and no growth inhibitory effect on the xenografts whether given alone or in combination with estradiol.

42. A method according to claim 41, wherein the composition is one which has no effect on xenografts of the estrogen receptor-positive and estrogen dependent MCF-7 (ATCC HTB-22) human breast cancer cell line in nude mice, as evidenced by the composition

having no growth supportive effect and no growth inhibitory effect on the xenografts whether given alone or in combination with estradiol, even where the composition is administered in a dose which is 10 or even 100 times higher than a dose giving, in the same strain of nude mice, a maximum uterus weight increase.

43. A method according to any of claims 28-42, wherein the estrogen deficiency-conditioned symptom or disease is selected from the group consisting of menopausal symptoms, dermatological disorders such as ageing of the skin, dryness of mucous membranes (e.g. vaginal and intestine), brain related disease such as Alzheimer's including other types of dementia, bone and joint related disease such as osteoporosis, osteochondrosis, osteoarthritis, rheumatoid arthritis, healing of bone fractures, and reduce in skeletal fractures and disease such as hyperlipidaemia, hypercholesterolaemia, arteriosclerosis.

44. A method according to claim 43, wherein the estrogen deficiency-conditions symptoms are menopausal symptoms.

45. A method according to any of claims 28-44, wherein the composition is a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

46. A method according to claim 45, wherein the composition is or contains *Cimicifuga Racemosa* extract.

47. A method according to claim 45, wherein the composition is a composition comprising *Cimicifuga Racemosa* plant parts.

48. A method according to claim 45, wherein the composition is a composition comprising SPP-001.

49. A method according to claim 46, wherein the composition is a composition containing one or more chemical compounds contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

50. A method according to claim 28, wherein the composition is combined with a drug which has a selective estrogen receptor modulating (SERM) activity.

ART 34 AMDT

Claims

1. Use of a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof, which induces a physiological estrogen-like effect without interacting with breast cancer cells, in particular without stimulating breast cancer cells, for the preparation of a medicament for the treatment of estrogen deficiency symptoms or diseases of a mammal suffering from or having a high risk of developing breast cancer.

2. Use according to claim 1, wherein the composition which induces a physiological estrogen-like effect without stimulating breast cancer cells.

3. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is uterine growth as determined by an increase in uterine weight compared to controls after administration of the composition to ovariectomized female athymic nude mice.

4. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is uterine growth as determined by an increase in mean uterine weight compared to controls of at least 0.10 g after administration of the composition to ovariectomized NMRI female athymic nude mice for 8 days.

5. Use according to any of claims 3 or 4, wherein the increase in uterine weight obtained by administration of a dose comparable to a normal dose for the mammal to be treated of the composition corresponds to a weight increase obtainable in the same test animal by estradiol treatment.

6. Use according to any of claims 3 or 4, wherein the increase in uterine weight obtained by administration of a dose comparable to a normal dose for the mammal to be treated of the composition corresponds to a substantially maximum weight increase obtainable in the same test animal by estrogen treatment.

7. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is a change in gonadotropins (FSH and/or LH) as determined by available validated radioimmuno assay techniques.

8. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is a change in cytology of the vaginal cells as determined by cytological counts.

Sub A4

9. Use according to any of the preceding claims, wherein the composition do not interact, in particular stimulate, cancer cells that are estrogen receptor-negative.

10. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the composition compared to a control on growth of the estrogen and progesterone receptor negative MDA-MB-231 (ATCC HTB-26) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

11. Use according to any of the preceding claims, wherein the composition do not interact, in particular stimulate, cancer cells that are estrogen receptor-positive.

12. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the composition compared to a control on growth of the estrogen dependent and estrogen receptor-positive MCF-7 (ATCC HTB-22) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

13. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the composition when given in combination with estradiol compared to a control on growth of the estrogen dependent and estrogen receptor-positive MCF-7 (ATCC HTB-22) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

14. Use according to any of the preceding claims for the treatment of estrogen deficiency symptoms or diseases of humans having breast cancer, having a high risk of recurrent breast cancer, or having a risk (such as high risk) of developing breast cancer.

15. Use according to any of the preceding claims, wherein the estrogen deficiency-conditioned symptom or disease is selected from the group consisting of menopausal symptoms; dermatological disorders such as ageing of the skin, wrinkles, dry skin and other estrogen deficiency related dermatological disorders; dryness of mucous membranes (e.g. vaginal and intestine); brain related disease such as Alzheimer's including other types of dementia; bone and joint related diseases such as osteoporosis, osteochondrosis, osteoarthritis, rheumatoid arthritis, healing of bone fractures, and reduction in skeletal fractures; vaginal estrogen deficiency such as vaginal dryness and dyspareuni; coronary heart diseases such as arteriosclerosis; and disease such as hyperlipidaemia and hypercholesterolaemia.

16. Use according to any of the preceding claims, wherein the estrogen deficiency-conditions symptoms are menopausal symptoms.

17. Use according to any of the preceding claims, wherein the composition is or contains *Cimicifuga Racemosa* extract.

18. Use according to any of the preceding claims, wherein the composition is a composition comprising *Cimicifuga Racemosa* plant parts.

19. Use according to any of the preceding claims, wherein the composition is a composition comprising SPP-001.

20. Use according to any of the preceding claims, wherein the composition is a composition containing one or more chemical compounds contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

21. Use according to any of the preceding claims, wherein the composition is combined with a drug which has a selective estrogen receptor modulating (SERM) actitivty.

22. A container comprising a composition according to any of the preceding claims with a pharmaceutically carrier and comprising an indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening estrogen dependent cancer.

23. A container comprising a composition which induces a physiological estrogen-like effect without stimulating breast cancer cells with a pharmaceutically carrier and comprising an indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening estrogen dependent cancer.

24. A method for relieving symptoms caused by estrogen deficiency in a mammal suffering from or having a high risk of developing an estrogen dependent tumour comprising administering to the mammal a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof, which induces a physiological estrogen-like effect without stimulating breast cancer cells.

25. A method according to the previous claim, wherein the mammal is a human.

26. A method for screening for substances or compositions which can be used according to claim 1 or 2, comprising subjecting test substances or compositions to

1) testing for possible estrogen-like effect in normal tissue by measuring increase in uterine weight, changes in gonadotropins, changes in vaginal cytology and/or post-menopausal symptoms in an adult female mammal and
2) testing for possible estrogenic effect in breast cancer, and selecting, as candidates for tissue-selective estrogenic substances or compositions useful in the method according to claim 1 or 2, substances or compositions which,
   a) are capable of inducing physiological estrogenic effects in female mammals, and at the same time
   b) have no effect on the growth of estrogen receptor-negative cancer cells and no effect on estrogen receptor-positive cancer cells in the doses in which they induce physiological estrogen effects.

27. A method according to claim 26, wherein the capability of the substance or composition of inducing physiological estrogen effects e.g. uterine growth female mammals is tested by testing the capability of the substance or composition of effecting uterine weight increase in ovariectomized female NMRI athymic nude mice, the lack of effect of the substance or composition on the growth of estrogen receptor-negative cancer cells is assessed as the lack of capability of the substance or composition of supporting growth of MDA-MB231 xenografts in female NMRI athymic nude mice, and the lack of effect of the substance or composition on the growth of estrogen receptor-positive cancer cells is as-

sessed as the lack of capability of the substance or composition of supporting growth of MCF-7 (ATCC (HTB-22) xenografts in female NMRI athymic nude mice.

28. A method for relieving or curing symptoms or diseases which are caused by estrogen deficiency, or which can be relieved or cured by administration of steroidal estrogen, in a mammal who suffers from breast cancer, or has a risk of recurrent breast cancer, or has a high risk of developing breast cancer,
the method comprising administering, to the mammal, a composition
which has an estrogen-like effect, as evidenced by a capability of the composition of inducing physiological estrogenic effects in adult mammal, and
which is free from interaction with breast cancer cells, in particular free from a stimulating effect on breast cancer,
thereby treating estrogen deficiency symptoms or diseases without introducing a risk of provoking the development of clinically evident breast cancer and/or stimulating growth of existing breast cancer cells in the mammal.

29. A method according to claim 28, wherein the mammal is female mammal.

30. A method according to claim 29, wherein the female mammal is a woman.

31. A method according to any of claims 29-30, wherein the estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing an increase in uterine weight in adult ovariectomized NMRI female athymic nude mice.

32. A method according to claim 31, wherein the increase in uterine weight following a dose comparable to a normal dose for the mammal to be treated corresponds to a weight increase seen in the same test animal following estradiol treatment.

33. A method according to claim 32, wherein the increase in uterine weight following a dose comparable to a normal dose for the mammal to be treated corresponds to a substantially maximum weight increase obtainable in the same test animal by estrogen treatment.

34. A method according to any of claims 28-33, wherein the estrogen-like

effect possessed by the composition manifests itself in the composition being capable of inducing a lowering in FSH and LH in females.

35. A method according to any of claims 30-34, wherein the estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing an estrogen like change in vaginal cytology in females.

36. A method according to any of claims 28-35, wherein the composition is one which has no effect on the growth of estrogen receptor-negative cancer cells.

37. A method according to claim 36, wherein the composition is one which has no effect on the growth of xenografts of the estrogen and progesteron receptor-negative MDA-MB-231 (ATCC HTB-26) human breast cancer cell line in nude mice.

38. A method according to any of claims 28-37, wherein the composition is one which is free from any effect on breast cancer cells even where the breast cancer cells are documented as being estrogen receptor-positive.

39. A method according to any of claims 28-38, wherein the composition is one which has substantially no agonizing and substantially no antagonizing effect on the effect of estrogen such as estradiol on breast cancer cells, even where the breast cancer cells are documented as being estrogen receptor-positive.

40. A method according to claim 39, wherein the composition is one which substantially does not bind to estrogen receptors of cancer cells.

41. A method according to any of claims 38-40, wherein the composition is one which has no effect on xenografts of the estrogen receptor-positive and estrogen dependent MCF-7 (ATCC HTB-22) human breast cancer cell line in nude mice, as evidenced by the composition having no growth supportive effect and no growth inhibitory effect on the xenografts whether given alone or in combination with estradiol.

42. A method according to claim 41, wherein the composition is one which has no effect on xenografts of the estrogen receptor-positive and estrogen dependent MCF-7 (ATCC HTB-22) human breast cancer cell line in nude mice, as evidenced by the composition

having no growth supportive effect and no growth inhibitory effect on the xenografts whether given alone or in combination with estradiol, even where the composition is administered in a dose which is 10 or even 100 times higher than a dose giving, in the same strain of nude mice, a maximum uterus weight increase.

43. A method according to any of claims 28-42, wherein the estrogen deficiency-conditioned symptom or disease is selected from the group consisting of menopausal symptoms, dermatological disorders such as ageing of the skin, dryness of mucous membranes (e.g. vaginal and intestine), brain related disease such as Alzheimer's including other types of dementia, bone and joint related disease such as osteoporosis, osteochondrosis, osteoarthritis, rheumatoid arthritis, healing of bone fractures, and reduce in skeletal fractures and disease such as hyperlipidaemia, hypercholesterolaemia, arteriosclerosis.

44. A method according to claim 43, wherein the estrogen deficiency-conditions symptoms are menopausal symptoms.

45. A method according to any of claims 28-44, wherein the composition is a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

46. A method according to claim 45, wherein the composition is or contains *Cimicifuga Racemosa* extract.

47. A method according to claim 45, wherein the composition is a composition comprising *Cimicifuga Racemosa* plant parts.

48. A method according to claim 45, wherein the composition is a composition comprising SPP-001.

49. A method according to claim 46, wherein the composition is a composition containing one or more chemical compounds contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

50. A method according to claim 28, wherein the composition is combined with a drug which has a selective estrogen receptor modulating (SERM) activity.

COMPOSITION HAVING STEROIDAL ESTROGEN EFFECT WITHOUT INCREASING THE RISK OF BREAST CANCER

Field of the invention

The present application relates to a pharmaceutical composition with steroidal estrogen effect. This composition, however, does not have the steroidal estrogen side-effect of increasing the risk of development or the tendency to relapse of breast cancer. The application further relates to the uses of such compositions and methods for identifying same.

Background of the invention

In women cessation of ovarian function is associated with various somatic and psychological disorders which are clinically summarised as menopausal symptoms. The most characteristic and frequent symptoms are cessation of menstrual bleeding, hot flushes, depression, nervousness and insomnia. In addition, many women will suffer from osteoporosis due to insufficient endogenous estrogen production. Menopausal symptoms are the result of substantially reduced steroid production of the ovaries. Thus, one possibility for treatment of menopausal symptoms is substitution treatment with ovarian hormones. Steroidal estrogens have been the treatment of choice, even though it is well known that this type of treatment may increase the risk of cancer development. In particular, for patients with a prior or a current breast cancer, it is advised not to use steriodal estrogens due the risk of stimulating dormant cancer cells.

An alternative to steroidal estrogens for the treatment of menopausal symptoms are plant estrogens (phytoestrogens). Among these, *Cimicifuga Racemosa* has been reported to be a successful therapeutic approach with beneficial effects on menopausal symptoms (1, 12-15, 20, 21). However, theoretically phytoestrogens would bind to estrogen receptors in target tissues including breast cancer cells in order to exert their estrogenic activity. It is therefore to be expected that phytoestrogens would stimulate growth of estrogen sensitive breast cancer cells. The study by Martin, P.M. et al. is in support of a growth stimulatory effect of plant estrogens on breast cancer cells as it is demonstrated herein that plant estrogens may interact with the estrogen receptors in human breast cancer cells in culture and thereby stimulate the growth of the cells (16). It would thus appear inadvisable to offer

these compounds to women with a history of breast cancer or with a high risk of developing this disease.

Cimicifuga Racemosa extract and certain substances which are found, *inter alia*, in *Cimicifuga Racemosa* extract, have been suggested as estrogenic principles also in the patent literature, confer, e.g., US Patent No. 5,830,887, WO 98/50026 and EP 0847755.

A large fraction of women with early stage breast cancer (stage 1 and stage 2) experience cure of their disease after initial surgical treatment. It is, however, difficult to clearly define which women are most likely to have their disease return. Approximately 70% of all primary breast cancers express estrogen receptors and are therefore potentially estrogen sensitive and dependent, i.e. the growth of the cancer cells is stimulated by or dependent on estrogens (17, 18). It is therefore routinely recommended that these patients do not use steroidal estrogenic compounds at any time after the surgical resection of the primary breast cancer.

Currently available steroidal estrogens, which are most often used in hormone replacement therapy, do not discriminate between tissues or cell types. Thus, steroidal estrogens being used in clinical practice today may, while they provide a positive effect on estrogen deficiency symptoms and diseases, also carry the potential of enhancing cancer risk in women since they can stimulate cellular proliferation in several types of tissues, most notably breast tissue. Women with a family history of breast cancer and thereby with increased risk of developing breast cancer are therefore advised not to use steroidal estrogenic compounds.

Thus, there is at present an unmet need for substances or compositions with a differential estrogenic effect, i.e. compounds which by their estrogenic effects would relieve menopausal symptoms and other estrogen deficiency-related symptoms, but without stimulatory effect on the development and/or growth of breast cancer.

Summary of the invention

The present invention relates to a method for relieving or curing symptoms or diseases which are caused by estrogen deficiency or which can be relieved or cured by administration of steriodal estrogen, in mammals, in particular female mammals, in particular

women, who suffer from breast cancer, or have a risk of recurrent breast cancer, or have a high risk of developing breast cancer.

Thus, in one aspect, the invention relates to a method for relieving or curing symptoms or diseases which are caused by estrogen deficiency, or which can be relieved or cured by administration of steroidal estrogen, in a mammal who suffers from breast cancer, or has a risk of recurrent breast cancer, or has a high risk of developing breast cancer, the method comprising administering, to the mammal, a composition which has an estrogen-like effect, as evidenced by a capability of the composition of inducing known physiological estrogenic effects in target tissues, and which is free from interaction with breast cancer cells, in particular free from a stimulating effect on breast cancer thereby treating the estrogen deficiency-conditioned symptom or disease without introducing a risk of provoking the development of clinically evident breast cancer and/or stimulating growth of existing breast cancer cells in the mammal.

This means that treatment of the menopausal symptoms or other diseases based on estrogen deficiencies can now be performed without introducing the risk of stimulating breast cancer cells which is associated with administration of steroidal estrogen to a female mammal who suffers from breast cancer, or has a risk of recurrent breast cancer, or has a high risk of developing breast cancer.

The composition may, in a presently preferred embodiment, be a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof. Thus, the composition may be or may contain *Cimicifuga Racemosa* extract (either in a liquid form or in the dry form). Doses of the extract may be from, e.g. 1 mg to 20 mg daily on the basis of the extract, such as 2-3 mg twice daily for a woman, and doses of other active principles than the *Cimicifuga Racemosa* extract described herein (SPP-001) can be assessed by the person skilled in the art based on the relative activity compared to SPP-001 extract, or based on the experiments explained in the following.

Another use of the invention is to combine the composition with various pharmaceutical compounds to broaden the estrogen like effect of these compounds without interfering with any simultaneous anti-estrogenic effects of the compounds on breast cancer cells. One example is raloxifene (methanone, [6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3-yl][4-(1-piperidinyl)ethoxy]phenyl]-hydrochloride) and associated compounds. Other ex-

amples are non-steroidal anti-estrogens such as tamoxifen, droloxifene. In addition the composition could also be combined with biphosphonates.

Based on the screening made possible through the present invention, other substances or compositions showing the valuable tissue-selective estrogenic effect may be identified.

Thus, another aspect of the invention relates to a method for screening for substances or compositions with the described properties comprising subjecting test substances or compositions to

1) testing for possible estrogen-like effect in normal tissue by measuring e.g. increase in uterine weight in an adult ovariectomized female rodent and
2) testing for possible estrogenic effect in breast cancer, and selecting, as candidates for tissue-selective estrogenic substances or compositions useful for relieving or curing symptoms or diseases which are caused by estrogen deficiency or which can be relieved or cured by administration of steriodal estrogen, in women, who suffer from breast cancer, or have a risk of recurrent breast cancer, or have a high risk of developing breast cancer, substances or compositions which,
   a) are capable of inducing physiological estrogen effects e.g. increase in uterine wet weight or changes in gonadotropins or changes in bone mineral density, in an adult ovariectomized female rodent, and at the same time
   b) have no effect on the growth of estrogen receptor-negative cancer cells and no effect on estrogen receptor-positive cancer cells in the doses in which they induce physiological estrogen effects, e.g. does not bind to the estrogen receptor in breast cancer cells.

This aspect of the invention is also explained in greater detail in the following, as are protocols for clinical work.

DETAILED DESCRIPTION OF THE INVENTION

In Example 1 the estrogenic effect of SPP-001 on mouse uterine tissue is described. The dose selected was based on the recommended daily intake of SPP-001 in women, which is 2.4 mg/kg twice daily.

The increase in uterine weight induced by SPP-001 corresponds to that obtained following treatment with estradiol. Thus, it can be concluded that SPP-001 has estrogen-like effect on murine uterine tissue and that the applied administration and dose of SPP-001 is sufficient to induce this effect.

Example 2 describes the effect of SPP-001 on tumour development and growth of an estrogen receptor-negative human breast cancer grown in nude mice. Using the same dose of SPP-001 which induced increase in uterine weight, no effect was observed on the human breast cancer xenograft.

In Example 3, the estrogen receptor-positive human breast cancer cell line MCF-7 (ATCC HTB-22) was inoculated into nude mice and treated with estradiol or SPP-001. The SPP-001 dose was either the same as used in Example 1 and 2 or a 10 fold higher dose. The MCF-7 human breast cancer only forms tumours in ovariectomized or intact female nude mice in the presence of estrogen supplementation. In accordance with this, estrogen supplementation resulted in the development of growing tumours, while untreated mice did not develop any tumours. Treatment with SPP-001 in both dose groups did not result in tumour development in any of the mice studied. These results suggest that SPP-001 has no estrogenic effect on estrogen receptor-positive breast cancer cells.

Example 4 describes the effect of orally administered SPP-001 and subcutaneously administered estradiol on tumour development and tumour growth of the estrogen receptor-positive and estrogen dependent MCF-7 human breast cancer xenograft in nude mice. SPP-001 dose was 100 fold higher than the recommended daily dose for humans. The treatment with SPP-001 and estradiol was given both alone and in combination. SPP-001 had no growth supportive effect or growth inhibitory effect whether given alone or in combination with E2, suggesting that SPP-001 has neither potentiating nor inhibitory effect on estrogen sensitive breast cancer cells. The results further imply that SPP-001 does not bind to estrogen receptors of the MCF-7 cells, in that it does not antagonize the growth stimulatory effect of estradiol.

Example 5 describes an experimental model system for the testing of compositions or compounds ("drugs") with putative differential estrogen-like effects in normal tissues and in breast cancer. Possible estrogen-like effects in normal tissues are tested by e.g. measuring increase in mouse uterine weight as the end point, whereas possible estrogenic ef-

fect in breast cancer is tested by measuring growth supportive effect in the estrogen sensitive MCF-7 breast cancer xenograft as the end point. The tests can be performed in the same mice or in identical mice.

Example 6 describes a clinical protocol for safety studies of SPP-001 treatment in women with advanced breast cancer. Patients are randomized to treatment with SPP-001 or placebo and will be followed until death. Survival curves for each group will be constructed in order to determine the possible effect of SPP-001 in this patient population. It will be understood that this protocol can be adapted for use for safety studies of treatment with any composition, CR-based or not, which fulfils the criteria for being useful in the method according to the invention.

Example 7 describes a clinical protocol for safety studies of SPP-001 treatment in women with advanced breast cancer and who have obtained a complete or partial response upon conventional antineoplastic therapy. Patients are randomized to treatment with SPP-001 or placebo and will be followed until death. Time to progression in each treatment group will be determined and compared in order to establish whether SPP-001 has any effect on cancer progression in this patient population. Again, it will be understood that this protocol can be adapted for use for safety studies of treatment with any composition, CR-based or not, which fulfils the criteria for being useful in the method according to the invention.

In example 8, a clinical protocol is described for safety studies of SPP-001 treatment in women with a prior diagnosis of breast cancer and with no indication of recurrence. Patients are randomized to treatment with SPP-001 or placebo and will be followed for 5 years. Univariate recurrence-free and overall survival curves will be constructed and compared in order to establish whether SPP-001 has any effect on these two parameters. Also this protocol can be adapted for use for safety studies of treatment with any composition, CR-based or not, which fulfils the criteria for being useful in the method according to the invention.

Example 9 describes clinical SPP-001 treatment of estrogen deficiency conditions other than menopausal symptoms in women with current breast cancer, with a prior diagnosis of breast cancer, and with an increased risk of developing breast cancer. The diseases include osteoporosis, osteochondrosis, hyperlipidaemia, hypercholesterolaemia, arteriosclerosis and other conditions which can be cured or relieved by estrogen replacement

therapy. It will be understood that the principles which appear from this example can be used with any other composition which fulfils the above mentioned criteria.

Thus a broad aspect of the present invention relates to a method for inducing a physiological estrogen-like effect without interacting with breast cancer cells and the use of a substance, capable of inducing a physiological estrogen-like effect without interacting with breast cancer cells, for the preparation of a medicament for the treatment of estrogen deficiency symptoms or diseases of a mammal suffering from or having a high risk of developing an estrogen dependent tumour.

The present invention is based on in vivo investigations performed on human breast cancer xenografts in immune deficient nude mice. Numerous studies have demonstrated that the responses of these breast cancer xenografts to endocrine treatment, i.e. ablative, inhibitive, additive, and competitive treatment are comparable to those obtained with these treatment modalities in breast cancer patients (2, 6-10). It is thus justified to presume that the response to SPP-001 of breast cancer *in situ* is comparable to the response obtained in the xenografts.

Estrogen has many physiological effects as estrogen is required for the normal maturation of the female e.g. by stimulating the development of the vagina, uterus, and uterine tubes as well as the secondary sex characteristics. Estrogen stimulates stromal development and ductal growth in the breast and is responsible for the accelerated growth phase and the closing of the epiphyses of the long bones that occur at puberty. Estrogen contributes to the growth of the axillary and pubic hair and alter the distribution of body fat so as to produce typical female body contours. Large quantities of estrogen also stimulate development of pigmentation in the skin most prominent in the region of the nipples and areolas and in the genital region. Estrogen also plays an important role in the development of the endometrial lining. Estrogen also have a number of important metabolic effects. It is responsible for the maintenance of the normal structure of the skin and blood vessels in women. Estrogen decreases the rate of resorption of bone by antagonizing the effect of parathyroid hormone on bone but do not stimulate bone formation. In the liver, Estrogen alters the metabolism so that there is a higher circulating level of proteins such as transcortin (CBG), thyroxinebining globulin (TBG), sex hormone-binding globulin (SHBG), transferring, renin substrate, and fibrinogen. This leads to increased circulating levels of e.g. thyroxine, estrogen, testosterone, iron, and copper. Preferred physiological effects

used as a measurement of consequence of treatment with the substance are stimulation of vaginal cells in, e.g., vaginal sm ar from a test animal, and measuring changes in gonadotropins, e.g. FSH and LH.

It will be obvious for the person skilled in the art to use one of the above mentioned physiological estrogen effects as a measure of estrogen-like effect for a substance. One of these physiological estrogen effects, the stimulation of uterine growth, have been chosen in the present technical teaching (examples 1-4). Thus, in a preferred embodiment of the invention the physiological estrogen-like effect is uterine growth as determined by an increase in uterine weight compared to controls after administration of the substance to ovariectomized female athymic nude mice. That is, a physiological estrogen-like effect is uterine growth as determined by an increase in mean uterine weight compared to controls of at least 0.10 g after administration of the substance to ovariectomized NMRI female athymic nude mice for 8 days.

The estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing an increase in uterine weight in adult ovariectomized NMRI female athymic nude mice, and for preferred compositions, the increase in uterine weight following a dose comparable to a normal dose for the mammal to be treated may correspond to a uterine weight increase seen in the same test animal following estradiol treatment, which will often be substantially the maximum uterine weight increase obtainable in the same test animal by estrogen treatment.

It is an important aspect of the invention that the substance is one which has substantially no effect on the growth of breast cancer cells, neither a positive, agonising, effect, nor a negative, antagonising, effect, and that this applies both for estrogen receptor-negative breast cancer cells (and also includes lack of indirect effect) and for estrogen receptor-positive breast cancer cells. The preferred lack of an antagonistic effect of the substance is based on the experimental reports demonstrating stimulation of breast and endometrial cancer cell growth following antiestrogen treatment of cells propagated in culture or athymic nude mice (22) and the often observed "flare" response to antiestrogens. It is preferred that the substance do not stimulate cancer cells that are estrogen receptor-positive. Likewise, it is preferred that the substance do not stimulate cancer cells that are estrogen receptor-negative. Furthermore, the substance should have no substantive effect on the breast cancer cells' capability of metastasizing or adhesion.

In one embodiment lack of stimulation of breast cancer cells is determined by no effect of the substance on growth of the estrogen and progesterone receptor negative MDA-MB-231 (ATCC HTB-26) human breast cancer cell line inoculated into six-week-old female athymic nude mice compared to a control wherein the tumours show increasing size during at least six consecutive growth recordings. Such method is also described in details in example 2.

In another embodiment lack of stimulation of breast cancer cells is determined by no effect of the substance on growth of the estrogen dependent and estrogen receptor-positive MCF-7 human breast cancer cell line inoculated into six-week-old female athymic nude mice compared to a control wherein the tumours show increasing size during at least six consecutive growth recordings. Such method is also described in details in example 3.

In a third embodiment lack of stimulation of breast cancer cells is determined by no effect of the substance when given in combination with estradiol on growth of the estrogen dependent and estrogen receptor-positive MCF-7 human breast cancer cell line inoculated into six-week-old female athymic nude mice compared to a control wherein the tumours show increasing size during at least six consecutive growth recordings. Such method is also described in details in example 4.

Ovariectomized rodents are suitable animal models for the study of estrogenic effects (2, 6-11). Administration of steroidal estrogens or other estrogenic compounds will result in increased uterine tissue weight. By using immune deficient rodents, the effects of steroidal estrogens and other estrogenic compounds can be studied simultaneously in the uterine tissue and in xenotransplanted human tumours.

The incidence of breast cancer is widespread. Women cured for breast cancer a still at risk of recurrent breast cancer, i.e. relapse. Quite a few women have a family history of breast cancer and are therefor at risk of developing breast cancer. Yet other women are in a position where they would prefer to minimise their discomfort during the menopause, without increasing their risk of breast cancer. Even though risk and high risk groups can be identified by statistical population studies, statistical grouping might not be relevant for the patient faced with the choice of minimising the discomfort during the menopause by

traditional estrogen treatment versus the increased their risk of breast cancer. The present substances will not put the female population in such unethical dilemma.

Thus based on the findings according to the invention, women who either suffer from breast cancer, or women who have suffered from breast cancer, and who in addition are suffering from menopausal symptoms, may due to the lack of estrogenic effects on breast cancer cells choose to use CR composition, e.g. SPP-001, or a substance according to the invention, composition to relieve their complains. Likewise, women who have a high risk of developing breast cancer due to a family history of breast cancer and/or due to carcinoma *in situ* lesions in their breast tissue, may choose to use CR composition e.g. SPP-001 to relieve their menopausal symptoms.

Thus, based on the findings according to the invention, women who either suffer from breast cancer, or women who have suffered from breast cancer, and who in addition are suffering from menopausal symptoms, may due to the lack of estrogenic effects on breast cancer cells choose to use CR compositions according to the invention, e.g. SPP-001, to relieve their complaints. Likewise, women who have a high risk of developing breast cancer due to a family history of breast cancer, genetic alterations related to an increase of breast cancer, nullipari, early menarche, late menopause, adipositas, prior use of steroidal estrogens, and carcinoma *in situ* lesion in their breast tissue, may choose to use CR composition, e.g. SPP-001 to relieve their menopausal symptoms.

In this context "high risk" is defined as a risk of developing breast cancer which is higher than 10% as compared to normal, i.e. a hazard ratio which is higher than 1,1 which is comparable to the increased risk of developing breast cancer when taking conventional contraceptive pills (hazard ratio of 1.1-1.2).

While by far the most important patient group to receive the treatment will be women, it is contemplated that the method of the invention may also be useful in human males, as it is also known that men may suffer from breast cancer and at the same time from symptoms or diseases which can be treated using estrogen.

As mentioned above, estrogen has a plethora of physiological effects. Thus, the symptoms of estrogen deficiency will vary. The substance according to the invention will consequently alleviate estrogen deficiency-conditioned symptom or disease selected from the

group consisting of menopausal symptoms; dermatological disorders such as ageing of the skin, wrinkles, dry skin and other estrogen deficiency related dermatological disorders; dryness of mucous membranes (e.g. vaginal and intestine); brain related disease such as Alzheimer's including other types of dementia; bone and joint related diseases such as osteoporosis, osteochondrosis, osteoarthritis, rheumatoid arthritis, healing of bone fractures, and reduction in skeletal fractures; vaginal estrogen deficiency such as vaginal dryness and dyspareuni; coronary heart diseases such as arteriosclerosis; and disease such as hyperlipidaemia and hypercholesterolaemia.

As the most common of these is menopausal symptoms, the treatment of these are preferred.

As illustrated in the examples, one substance that is capable of inducing a physiological estrogen-like effect without stimulating growth of breast cancer cells is *Cimicifuga Racemosa* extract (CR). Thus, a preferred substance according to the invention is a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof. Preferably a *Cimicifuga Racemosa* extract SPP-001.

Thus the invention is specifically based on the novel finding that a specific extract of CR (Cimicifuga racemosa) gives rise to a specific composition (SPP-001) which shows a unique combination of a very pronounced estrogenic effect, as assessed by support of uterus growth, and, in doses which give a maximum increase in uterus growth, comparable to the best increases obtainable by treatment with estradiol, shows a lack of estrogenic effects of SPP-001 on human breast cancer cells, hormone-sensitive and -dependent as well as hormone-resistant and -independent breast cancer cells. At present, it is not known whether this very beneficial combination is ascribable to one substance among the substances contained in the extract (some of which, such as formononetin, actein, 27-doxoactein, have been identified), but it seems likely that the effect is the effect of combinations of several substances in the extract, although it cannot be ruled out that it will be possible, using the principles for screening etc. which constitute an aspect of the present invention, to identify a single known or novel compound capable of exerting the valuable tissue-selective estrogenic effect.

In one aspect, the treatment according to the invention is based on extracts of *Cimicifuga Racemosa* (CR), which have known beneficial effects on menopausal problems in women

(1, 12-15, 20, 21). Such extracts may be water soluble and/or substantially soluble in organic solvent systems, e.g., by alcohol extraction of *Cimicifuga Racemosa* material, in particular such as root stem. The estrogen-like effect of CR is further documented by the expected chances in gonadotropins following CR treatment (12, 14).

The composition may also be a composition comprising *Cimicifuga Racemosa* plant parts, or it may be a composition containing one or more chemical compounds contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

The composition may also be used in combination with a drug which has a selective estrogen receptor modulating (SERM) activity. Such drugs having SERM activity may be raloxifene (methanone, [6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3-yl][4-(1-piperidinyl)ethoxy]phenyl]-hydrochloride) and associated compounds. Other examples are non-steroidal anti-estrogens such as tamoxifen, droloxifene. In addition the composition could also be combined with biphosphonates.

The composition to be used according to the invention may be an oral composition or a composition suitable for any other appropriate route of administration, and examples of suitable pharmaceutical preparations can, e.g., be found in the literature, including the above-mentioned patent literature.

Another embodiment of the invention relates to a container comprising a substance capable of inducing a physiological estrogen-like effect without stimulating breast cancer cells with a pharmaceutically carrier and comprising an indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening breast cancer.

In this context the term "container" should be given its broadest meaning to enclose all objects suitable to contain a pharmaceutical or naturopathic substance.

The indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening breast cancer can take any form. One preferred form is a label on the pill container. Such label could e.g. describe the estrogen-like effect of the substance and pointing to the lack of effect on breast cancer cells.

Another preferred form of the indication is a market authorisation of the substance wherein breast cancer is not contraindicated. Such authorisation could e.g. describe the estrogen-like effect of the substance and pointing to the lack of effect on breast cancer cells.

The present requirements for obtaining authorisation for sale of a herbal medicinal product are proof that the product does not have any negative impact on the human body. With the scientific findings described in the present application, a substance as defined herein, especially *Cimicifuga Racemosa* extracts e.g. SPP-001, is free from negative impact on cancer cells. In a preferred embodiment, the indication is a market authorisation of a herbal medicinal product stating the lack of effect on breast cancer cells.

Based on the foregoing it is preferred that the container comprises an extracted from *Cimicifuga Racemosa*.

If an estrogenic substance or composition could be identified which does exert estrogenic effects on menopausal symptoms and other symptoms or diseases caused by estrogen deficiency or relievable or curable by administration of estrogens, but without any influence on the growth of breast cancer cells, even estrogen-sensitive breast cancer cells, this substance or composition might find wide-spread use as a safe drug in hormone substitution therapy by women in general suffering from estrogen deficiency syndromes or diseases, especially women with a history of breast cancer, women with current breast cancer, and in women with a high risk of developing breast cancer.

As indicated above, the findings according to the invention and the models developed by the inventors for assessing the suitability of a composition or a compound as a tissue-selective estrogenic substance open up the possibility of finding other substances, compositions or chemical compounds which include the valuable tissue-selective estrogenic effect. Thus, one aspect of the invention relates to a method for screening for substances or compositions which are capable of inducing a physiological estrogen-like effect without interacting with breast cancer cells, comprising subjecting test substances or compositions to

1) testing for possible estrogen-like effect in normal tissue by measuring increase in uterine weight, changes in gonadotropins, changes in vaginal cytology and/or postmenopausal symptoms in an adult female mammal and

2) testing for possible estrogenic effect in breast cancer, and selecting, as candidates for tissue-selective estrogenic substances or compositions useful for relieving or curing symptoms or diseases which are caused by estrogen deficiency, or which can be relieved or cured by administration of steroidal estrogen, in a mammal who suffers from breast cancer, or has a risk of recurrent breast cancer, or has a high risk of developing breast cancer, substances or compositions which,
   a) are capable of inducing physiological estrogenic effects in female mammals, and at the same time
   b) have no effect on the growth of estrogen receptor-negative cancer cells and no effect on estrogen receptor-positive cancer cells in the doses in which they induce physiological estrogen effects.

It is preferred that the capability of the substance or composition of inducing physiological estrogen effects e.g. uterine growth female mammals is tested by testing the capability of the substance or composition of effecting uterine weight increase in ovariectomized female NMRI athymic nude mice, the lack of effect of the substance or composition on the growth of estrogen receptor-negative cancer cells is assessed as the lack of capability of the substance or composition of supporting growth of MDA-MB231 xenografts in female NMRI athymic nude mice, and the lack of effect of the substance or composition on the growth of estrogen receptor-positive cancer cells is assessed as the lack of capability of the substance or composition of supporting growth of MCF-7 xenografts in female NMRI athymic nude mice. Furthermore, the substance or compound should not support the growth of breast cancer cells even if combined with estradiol and even if the compound or substance is given in doses which are 10 or even 100 times higher than a dose giving, in the same strain of nude mice, a maximum uterus weight increase.

Examples

Example 1: EFFECT OF ORALLY ADMINISTERED SPP-001 ON UTERINE WEIGHT IN MICE

This example describes the measurement of physiological estrogenic effects of orally administered SPP-001 in mice. As an example effect of orally administered SPP-001 on uterine weight in adult ovariectomized NMRI female athymic nude mice are given.

Ovariectomy, randomization and identification

After acclimatization the animals were ovariectomized and randomized to two treatment groups and a group of untreated controls (see below). The animals and cages were marked accordingly.

Study design

The mice (10 weeks old NMRI mice) were ovariectomized and randomized at day 0. One group of mice served as untreated controls (n=5), and one group of mice (n=5) was given estradiol from day 1 to 8 by insertion of a 0.72 mg subcutaneous E2 pellet. In the last group the mice (n=6) were given SPP-001 orally twice daily from day 1 to 8.

Observations

The animals were observed daily during the experimental period of 8 days.

CR extraction and dosing procedure

For extraction a standardised CR material as for example provided by Finzelberg AG, Germany (catalogue number 0472 312 or 0472 340) was used.

The desired extraction solution was made from 100 mg CR material and 1660 ml sterile water, vortexed and then kept at room temperature for 30 minutes. This final CR composition was named SPP-001. SP- 001 was made freshly for each experimental day and al-

ways stored at +4°C for a maximum of 24 hours. For each day of the experiment the above described procedure was repeated.

Dosing was performed orally with a dosing volume of 0.1 ml of SPP-001 to each mouse twice daily. Each mouse in the SPP-001 group received approximately 0.40-0.60 mg SPP-001 material/kg/day.

Estradiol (E2) treatment procedure

One day after ovariectomy, a 0.72 mg estradiol slow release pellet (Innovative Research, Toledo, USA, cat. no. SE-121) was inoculated subcutaneously in the neck of the mice using a trocar.

Termination of experiment

The experiment was terminated at day 8 after ovariectomy. All animals were sacrificed by cervical dislocation and the mouse uterine weights were determined.

Results

The calculated mean uterine weights and the corresponding ranges were 0.08 g [0.05 - 0.11], 0.13 g [0.11 - 0.15], and 0.14 g [0.11 - 0.21] in the control, estradiol and SPP-001 groups, respectively (*Table 1* and *Figure 1*).

Table 1

Individual uterine weights (g)		
Untreated controls	Estradiol (E_2)	CR composition
0.09	0.11	0.14
0.11	0.15	0.11
0.08	0.14	0.13
0.06	0.12	0.21
0.05	0.15	0.11
		0.12

Effect of CR composition on uterine weight of ovariectomized adult NMRI/Bom™ female athymic nude mice.

CR composition was given orally, 0.24 mg/kg twice daily. E_2 treatment was given by subcutaneous inoculation of a 0.72 mg slow release pellet (Innovative Research) in the neck.

Discussion

The SPP-001 dose was chosen to simulate the daily recommended dose.

SPP-001 had an *in vivo* effect on mouse uterine weight which was comparable to that seen following E2 treatment. Other methods to determine physiological estrogenic activity such as changes in gonadotropins (decrease) and changes in cytology of the vaginal cells (maintaining normal histological profile) can be used either in addition or as an alternative.

Example 2: EFFECT OF ORALLY ADMINISTERED SPP-001 ON TUMOUR DEVELOPMENT AND GROWTH OF THE MDA-MB-231 HUMAN BREAST CANCER XENOGRAFT

This example describes the effect of orally administered SPP-001 on tumour development and tumour growth of the estrogen receptor-negative and estrogen resistant and independent MDA-MB-231 human breast cancer xenograft in nude mice (4).

Cells

The human mammary cancer cell line MDA-MB-231 (ATCC HTB-26) was used. Near confluent *in vitro* grown cells were harvested using a cell scraper. After centrifugation an aliquot of the cells was stained by Trypan Blue and counted. The cells were resuspended in fresh medium to a final concentration of 1×10^6 viable cells per inoculum (0.2 ml).

Animals

The experiment was performed in a total of 21 six-week-old female META/Bom™ athymic nude mice. An acclimatization period of one week was allowed in order to exclude animals in poor condition.

The mice were kept under sterile conditions in laminar air flow clean benches. Type III Macrolon cages (42 x 26 x 15 cm) with five animals in each cage was used. The mice were allowed sterile water and food pellets *ad libitum*.

The cages and the bedding were changed once a week. The room temperature was 25 ± 2°C, and the relative humidity 55 ± 5%. The room was illuminated 24 hours a day.

Animal randomization and identification

After acclimatization, the animals were randomized to a group of 11 treated animals and a group of 10 control mice. Each animal was identified by earmarks and each cage was marked to identify group and animal earmarks.

Study design

At day 0 the animals (n=21) were inoculated with tumour cells subcutaneously in both flanks.

The mice were then randomized into either treatment with SPP-001 (example 1) or treatment with sterile water. The mice of the SPP-001 treatment group were given SPP-001 orally (0,1 ml) twice daily from the day of cell inoculation and until termination of the experiment. Control mice received sterile water twice daily.

CR extraction and dosing procedure

As described in example 1.

Observations

The animals were observed daily during the experimental period of 38 days.

When the tumours became measurable they were measured in two dimensions three times a week using a sliding gauge. Tumour growth curves were constructed and growth curve parameters were calculated (19).

Tumours that did not grow during the entire experimental period and tumour inocula without take were excluded from the analysis.

Termination of experiment

The experiment was terminated when treated tumours had shown increasing size during at least six consecutive growth recordings. At termination of the experiment, the animals were sacrificed by cervical dislocation.

Results

No visible signs of toxicity was seen in animals given SPP-001.

When this experiment was terminated, a total of 31 tumours in 17 mice were evaluable. 15 tumours developed in control mice and 16 tumours developed in the treated mice. *Figures 2* show the mean tumour area growth curves constructed from the individual tumour measurements. Calculated tumour growth curve parameters are listed in *Table 2.*

The specific growth rate, α (*Table 2*) of treated tumours was 0.0144 compared to 0.0165 of the vehicle treated control tumours, and as a consequence the calculated tumour volume doubling times (TD) were 14.9 days and 13.0 days of the treated and control tumours, respectively.

Tabl 2

Group	Growth curve analysis				
	Tumours[1] No.	Gompertz growth curve parameters[2] $\alpha \times 10^3$	$\beta \times 10^3$	r	T_D[3] days
Controls	15	16.5	96.3	0.992	13.0
CR composition	16	14.4	80.3	0.994	14.9

Growth analysis of human MDA-MB-231 breast carcinoma xenografts grown in CR composition treated and untreated control female META/Bom™ athymic nude mice.

[1] Number of evaluable tumours; inocula without growth were excluded.

[2] The data on tumour area (product of two perpendicular measurements) were described according to a Gompertz function.

$$A(t) = A(0)\exp\{(1-\exp(-\alpha t))\beta/\alpha\}$$

A(0) is the tumour area at the time of tumour cell inoculation, A(t) is the tumour area at time t after inoculation, and α and β are constants determining the course of the growth curves.

By linear regression the growth data were fitted to a straight lined transformation of the Gompertz function.

$$\ln[\ln A(max) - \ln A(t)] = \ln(\beta/\alpha) - \alpha t$$

A(max) is the theoretical maximal tumour area, α represents the slope, and r is the coefficient of correlation.

Tumour volume doubling time, T_D, was computed from the Gompertz parameters. Tumour volume was calculated from

$$V(t) = \Pi/6\, A^{3/2}\, k,$$

where A is tumour area, and k is a previously established constant of the relation between the two measurements obtained and the third dimension of the tumours.

Discussion

The results of the growth curve analysis indicate that the applied dose of SPP-001 had no effect on growth of the estrogen and progesterone receptor negative MDA-MB-231 human breast cancer cell line. Similar experiments as those described in this example can be performed using other estrogen receptor-negative human breast cancer cell lines, e.g. MDA-MB-435S (ATCC HTB 129).

Example 3: EFFECT OF ORALLY ADMINISTERED SPP-001 ON TUMOUR DEVELOPMENT AND GROWTH OF THE MCF-7 HUMAN BREAST CANCER XENOGRAFT

This example describes the effect of orally administered SPP-001 on tumour development and tumour growth of the estrogen receptor-positive and estrogen dependent MCF-7 human breast cancer xenograft in nude mice (4).

Cells

The estrogen and progesterone receptor positive human mammary cancer cell line MCF-7 (ATCC HTB-22) was used.

Near confluent *in vitro* grown cells were harvested using a cell scraper. After centrifugation an aliquot of the cells was stained by Trypan Blue and counted to determine the fraction of viable cells. The cells were resuspended in fresh medium to a final concentration of 10^6 - 10^7 viable cells per inoculum (0.1-0.2 ml). The cells were inoculated subcutaneously into both flanks of intact or ovariectomized female nude mice.

In separate experiments, xenotransplanted tumours in intact female nude mice were used as the source for the experiments. 1-2 mm diameter tumour blocks were prepared and inoculated into the right flank of recipient mice.

Animals

The experiment was performed in a total of 80 six-week-old female NMRI/Bom™ athymic nude mice. An acclimatisation period of one week was allowed in order to exclude animals in poor condition.

After acclimatisation some of the animals were ovariectomized under general anaesthesia and using standard procedures. Transplantation of tumours and inoculation of cells were performed following at least one week to ensure full recovery after the ovariectomy.

The mice were kept under sterile conditions in laminar air flow clean benches. Type III Macrolon cages (42 x 26 x 15 cm) with five animals in each cage were used. The mice were allowed sterile water and food pellets *ad libitum*.

The cages and the bedding were changed once a week. The room temperature was 25 ± 2°C, and the relative humidity 55 ± 5%. The room was illuminated 24 hours a day.

Animal randomization and identification

After inoculation of cells or tumour blocks the animals were randomized into treatment groups according to the study design. Each animal was identified by earmarks and each cage was marked to identify group and animal earmarks.

Study design

Three series of experiments were performed.

Study 1 Forty ovariectomized animals were inoculated with 10^6 MCF-7 cells, and the mice were randomized into four treatment groups: Untreated controls, 0.40-0.60 mg/kg/day SPP-001, 4 - 6 mg/kg/day SPP-001, and 0.72 mg estradiol slow release pellet (Innovative Research).

Study 2 Twenty intact female nude mice were inoculated with 10^7 MCF-7 cells, and the mice were randomized into two treatment groups: 4 - 6 mg/kg/day SPP-001 or 0.72 mg estradiol slow release pellet (Innovative Research).

Study 3 Twenty intact female nude mice were inoculated with tumour blocks of MCF-7 xenografts into the right flank of recipient mice, and the mice were randomized into two treatment groups: 4 - 6 mg/kg/day SPP-001 or 0.72 mg estradiol slow release pellet (Innovative Research), respectively.

CR extraction and dosing procedure

Study 1. 0,1 ml twice daily of SPP-001 (as given in example 1) was given.

Study 2-3. 0,1 ml twice daily of a concentrated SPP-001 (1/10 volume water was used as described in example 1) was given.

Estradiol (E2) treatment procedure

At the day of inoculation of cells or tumour blocks a 0.72 mg estradiol slow release pellet (Innovative Research) was inoculated subcutaneously in the neck of the mice using a trocar.

Observation & calculations

The animals were observed daily during the experimental periods. When the tumours became measurable, they were measured in two dimensions three times a week using a sliding gauge. Tumour growth curves were constructed and growth curve parameters were calculated (19).

Termination of experiment

The experiment was terminated when growing tumours had shown increase in size during at least six consecutive growth recordings. At termination of the experiment, the animals were sacrificed by cervical dislocation.

Results

In non of the three studies toxicity was observed.

Study 1. The experiment was terminated 27 days after initiation because of a very low take rate. No tumours developed in the group of untreated controls or in the two treatment groups. However, only in one of the 10 E2 treated mice a growing tumour appeared.

Study 2. In this experiment six out of 9 evaluable E2 treated mice developed a total of 10 growing MCF-7 tumours. In contrast, in 10 out of 10 evaluable SPP-001 treated animals no tumours developed.

Study 3. When this experiment was terminated 52 days after transplantation, tumours had developed in 7 out of 10 mice in the E2 treatment group. No tumours were detected in 10 mice treated with the SPP-001.

Figure 3 shows the mean tumour area growth curve constructed from the individual tumour measurements of the E2 treated tumours, and the lack of tumour growth in SPP-001 treated mice is indicated in the figure. Calculated tumour growth curve parameters are listed in *Table 3*.

Table 3

Group	Growth curve analysis				
	Tumours No.	Gompertz growth curve parameters $\alpha \times 10^3$	$\beta \times 10^3$	r	T_D days
Estradiol (E_2)	7/10	7.6	34.6	0.903	28.3
CR composition	0/10	-	-	-	-

Growth analysis of human MCF-7 breast carcinoma xenografts grown in CR composition and E_2 treated female NMRI/Bom™ athymic nude mice.

Discussion

The presented series of experiments showed that the applied SPP-001 did not have tumour stimulatory effect on estrogen dependent MCF-7 human breast carcinoma xenografts. In contrast , treatment with E2 supported the growth of this tumour, thus confirming the ability of MCF-7 to form tumours in nude mice under adequate hormonal substitution. Similar experiments as those described in this example can be performed using other estrogen receptor-positive human breast cancer cell lines, e.g. T47D and ZR75-1 (5).

The results of *Study 2* and *3* were identical in the observed tumour growth despite the difference in method of tumour establishment, i.e. *in vitro* grown single cell suspensions *vs.* solid tumour blocks.

Although tumour growth was only observed in the E2 treatment group of *Study 1*, the results of this experiment was inconclusive due to the low take rate. This was probably

caused by a relatively low cell dose of the inocula (10^6 cells). Therefore the cell dose was increased to 10^7 cells/inoculum in *Study 2*, resulting in a take rate comparable to that obtained with transplantation of tumour blocks in *Study 3*.

The SPP-001 dose of 0.40-0.60 mg/kg/day was chosen to simulate the recommended daily intake of CR. The results showed that even a 10-fold increase in the dose of SPP-001 did not support the growth of MCF-7.

The MCF-7 breast carcinoma in nude mice is a slow growing tumour (3). The specific growth rate, $\alpha = 0.0076$ (*Table 3*) corresponds to a tumour volume doubling time (TD) of 28.3 days.

Example 4: EFFECT OF CONCOMITANT ORAL SPP-001 AND SUBCUTANEOUS ESTROGEN TREATMENT ON TUMOUR DEVELOPMENT AND TUMOUR GROWTH

This example describes the effect of orally administered SPP-001 and subcutaneously administered estrogen on tumour development and tumour growth of the estrogen receptor-positive and estrogen dependent MCF-7 (ATCC HTB-22) human breast cancer xenograft in nude mice (4).

Cells

The estrogen and progesterone receptor positive human mammary cancer cell line MCF-7 was used.

Near confluent *in vitro* grown cells were harvested using a cell scraper. After centrifugation an aliquot of the cells was stained by Trypan Blue and counted to determine the fraction of viable cells. The cells were resuspended in fresh medium to a final concentration of 10^6 - 10^7 viable cells per inoculum (0.1-0.2 ml). The cells were inoculated subcutaneously into both flanks of intact female nude mice. One resulting MCF-7 tumour was used for serial transplantation into all the mice of this study.

Animals

The experiment was performed in a total of 32 six-week-old female NMRI/Bom™ athymic nude mice. An acclimatization period of one week was allowed in order to exclude animals in poor condition.

The mice were kept under sterile conditions in laminar air flow clean benches. The mice were allowed sterile water and food pellets *ad libitum*. The cages and the bedding were changed once a week. The room temperature was 25 ± 2°C, and the relative humidity 55 ± 5%.

Animal randomization and identification

After inoculation of tumour blocks the animals were randomized into treatment groups according to the study design. Each animal was identified by earmarks and each cage was marked to identify group and animal earmarks.

Study design

Thirty-two intact female nude mice were transplanted with approximately 1-mm-diameter blocks of MCF-7 tumour into the both flanks of recipient mice, and the mice were randomized into four treatment groups:

1: 40-60 mg/kg/day SPP-001 orally.
2: 0.72 mg slow release estradiol pellet s.c. (Innovative Research).
3: 40-60 mg/kg/day SPP-001 and 0.72 mg estradiol slow release pellet (Innovative Research).
4: Sterile water p.o. twice daily.

CR extraction and dosing procedure

As described in example 1 except that a 100X dose was used.

Estradiol (E2) treatment procedure

At the day of inoculation of tumour blocks a 0.72 mg estradiol slow release pellet (Innovative Research) was inoculated subcutaneously in the neck of the mice using a trocar.

Observation & calculations

The animals were observed daily during the experimental period. When the tumours became measurable, they were measured in two dimensions three time a week using a sliding gauge. Tumour growth curves were constructed and growth curve parameters were calculated (19).

Termination of experiment

The experiment was terminated when the growing had shown increase in size during at least six consecutive growth recordings. At termination of the experiment, the animals were sacrificed by cervical dislocation.

Results

No toxicity was observed.

As expected, the untreated control tumours showed no significant growth. At day 20 after transplantation 10 persisting tumour inocula were evaluable (*Figure 4*). The SPP-001 treatment did not support the growth of the MCF-7 tumour. The mean tumour area of 5 evaluable tumour inocula at 20 days after transplantation was the same as that of the control group.

The E2 treatment had growth supportive effect on the MCF-7 tumour xenograft. In this group, 8 tumours showed growth, and at day 20 the tumours had reached a mean tumour area of 39 sq mm (*Figure 4*). The addition of the SPP-001 to E2 did not influence the growth of the tumour. At 20 days after transplantation, the mean tumour area of 11 evaluable tumours in the combined treatment group was the same as that of the E2 group.

Discussion

This study shows that even a 100 fold increase of the SPP-001 did not induce tumours in mice inoculated with hormone receptor positive human breast cancer cells. In contrast, the positive control treatment with E2 has the expected growth supportive effect on the estrogen sensitive and dependent MCF-7 breast cancer xenograft.

In the group where mice received both SPP-001 and estrogen treatment no differences were seen when compared to the estrogen treatment only. This suggests that SPP-001 neither has a potentiating nor an inhibitory (antagonizing) effect on estrogen sensitive breast tumours. The observation further implies that this SPP-001 does not bind to estrogen receptors in MCF-7 cells.

Example 5: EXPERIMENTAL MODEL FOR TESTING DIFFERENTIAL ESTROGENIC EFFECTS

This example describes an experimental model system for the investigation of drugs or combination of drugs with putative differential estrogenic effects in normal tissues and in breast cancer.

Normal tissue

The possible estrogenic effects on normal tissues are tested using increase in uterine weight as the end point. Alternatively, changes in gonadotropins or vaginal cytology could be used.

The investigation includes an estradiol treatment group which serves as a functional control of the study system.

Vaginal Cytology

Specimen: Smear, collected from vaginal vault or lateral wall, is spread onto pre-labelled glass slides and promptly spray- or wet-fixed.

Method: Papanicolaou staining and microscopy.
Application: Investigation of vaginal bleeding or discharge; follow up smears after previous cervical or endometrial dysplasia or following hysterectomy for malignancy. Cyto-hormonal evaluation is occasionally helpful in assessing hormonal status.

Interpretation: Cytological examination will detect dysplasia, neoplasia, and some infections e.g. infection with Trichomonas vaginalis or Candida albicans.

Animals

Six-eight-week-old female NMRI/Bom™ athymic nude (*nu/nu*) mice are used. After acclimatization the mice are ovariectomized in order to avoid influence of endogenous estrogen. The mice are randomised into treatment groups and a group of untreated controls.

The experiments described in this example can be performed with other strains of mice.

Study design

The mice are ovariectomized and randomised at day 0. One group serves as untreated controls (n=5-10), and one group (n=5-10) are given estradiol (E2) from day 1 by insertion of a 0.72 mg subcutaneous slow release E2 pellet (Innovative Research) in the neck of the mice using a trocar.

The last group(s) (n=5-10 in each group) are treated with the test composition(s)/drug(s) using relevant dose(s) and schedule(s).

Observations

The animals are observed daily during the experimental period of 8 days. Animals dying before termination of the experiment are excluded from the analysis.

Dosing procedure of test drug(s)

Composition(s) of the drug(s) are prepared using appropriate methods for extraction, purification, solubilisation and vehicle systems. The treatment is given by the most convenient route of administration (dermally, orally, intraperitoneally, or intravenously) and with appropriate dosing volume(s).

Dose(s) of the drug(s) are selected in relevant dose range(s), and schedule(s) with expected higher efficiency are selected.

Termination of experiment

The experiment is terminated at day 8 after ovariectomy. All animals are sacrificed by cervical dislocation and the mouse uterine weights are determined.

Evaluation

The mouse uterine weight data are used to calculate mean uterine weights of the control, estradiol, and test groups, respectively.

A significant increase in mean uterine weight of the E2 group compared to the controls serves as functional control of the study system.

A significant increase in mean uterine weight of the test drug(s) treatment group(s) are considered evidence of estrogenic effect on normal tissues.

Comparison of the increase in mean uterine weight in the E2 group and test group(s) are used to semiquantitate the estrogenic effect of the investigated drug(s), dose(s), and schedule(s).

Breast cancer

The possible estrogenic effects on breast cancer cells are tested using growth stimulation of an estrogen sensitive human breast cancer xenograft as the end point.

The investigation is performed in two human breast cancer xenografts using nude mice identical to those used for the uterine weight investigation. One tumour, MDA-MB-231 is estrogen and progesterone receptor negative, and therefore estrogen resistant and independent (4). The other tumour, MCF-7 is estrogen and progesterone receptor positive, and estrogen sensitive and dependent (4). The reason for inclusion of the receptor negative breast tumour xenograft in the test system is to identify possible unspecific effects, i.e. non-estrogenic effects which are independent of binding of the drug(s) to estrogen receptors of the tumour cells.

The investigation includes estradiol treatment of the estrogen sensitive MCF-7 tumour xenografts which serves as a functional control of the study system.

Tumour cells

The human mammary cancer cell lines MDA-MB-231 (ATCC HTB-26) and MCF-7 (HTB-22) are used. Near confluent *in vitro* grown cells are harvested using a cell scraper. After centrifugation an aliquot of the cells is stained by Trypan Blue and counted to determine the fraction of viable cells. The cells are resuspended in fresh medium to a final concentration of 10^6 - 10^7 viable cells per inoculum (0.1-0.2 ml). The cells are inoculated subcutaneously into both flanks of ovariectomized or intact female nude mice.

Animals

Six-week-old female athymic NMRI/Bom™ nude (*nu/nu*) mice are used. An acclimatization period of one week is allowed in order to exclude animals in poor condition. The experiments described in this example can be performed with other strains of mice.

Ovariectomy is performed under general anaesthesia and using standard procedures following acclimatization of the animals.

In ovariectomized mice inoculation of tumour cells is performed after at least one week to ensure full recovery after the ovariectomy.

After inoculation of cells the animals are randomized into treatment groups according to the study design.

The mice are kept under sterile conditions in laminar air flow clean benches. The mice are allowed sterile water and food pellets *ad libitum*. The room temperature is 25 ± 2°C, and the relative humidity 55 ± 5%.

Study design

The growth experiments with the two tumours are performed in separate series using similar study design.

At day 0, the animals are inoculated with tumour cells subcutaneously in both flanks. The mice are then randomized into one group of untreated (vehicle) controls (n=10) and a group (n=10) given E2 from day 1 by insertion of a 0.72 mg subcutaneous slow release

E2 pellet (Innovative Research) in the neck of the mice using a trocars. A third group(s) (n=10 in each group) is treated with test drug(s) using relevant dose(s) and schedule(s). The E2 treatment group is only included in the treatment of MCF-7 xenograft.

The dosing procedures are planned as described in the uterine weight section

The animals are observed daily during the experimental period. When the tumours become measurable they are measured in two dimensions three times a week using a sliding gauge. Tumour growth curves are constructed and growth curve parameters are calculated (19).

The experiment is terminated when growing tumours have shown increasing size during at least six consecutive growth recordings. At termination of the experiment, the animals are sacrificed by cervical dislocation.

Evaluation

Using a computer program according to Rygaard et al (19) the tumour measurements are used to construct mean growth curves and to calculate tumour growth curve parameters (19).

Stimulatory effect of E2 on the MCF-7 xenografts serves as functional control of the study system.

A growth supportive effect of the drug(s) in the MCF-7 xenograft indicates an estrogen-like effect mediated through estrogen receptors of the cancer cells. If a growth supportive effect of the drug(s) is also found in the MDA-MB-231 xenograft, this is considered evidence that the effect observed is not mediated through the estrogen receptors of the tumour cells. However, in order to be chosen as a candidate for a substance or composition useful in the method of the invention, the substance or composition should show neither growth supportive effect in the MCF-7 xenograft nor growth supportive effect (which would then be an indirect growth supportive effect) in the MDA-MB-231 xenograft.

Comparison of the growth supportive effect in the E2 group and test group(s) are used to semiquantitate the estrogenic effect of the investigated drug(s), dose(s), and schedule(s) according to Rygaard et al (19).

Similar experiments as those described in this example can be performed using other estrogen receptor-positive and negative human breast cancer cell lines, e.g. T47D, ZR75-1, and MDA-MB-435S (5).

Example 6: CLINICAL EVALUATION OF THE SAFETY OF SPP-001 IN WOMEN WITH ADVANCED BREAST CANCER

This example describes a clinical protocol for safety studies of SPP-001 in women with advanced breast cancer.

Patients

Advanced breast cancer patients with measurable disease will be included. The patients should suffer from menopausal symptoms. No chemotherapy or endocrine therapy should be given concomitantly with the SPP-001 treatment. Estrogen receptor (ER) status on the cancer cells should be determined.

Treatment

The SPP-001 should be administered orally twice daily at a corresponding dose of 0.40-0.60 mg SPP-001 /kg. Treatment period should be 3 months.

Study design

Patients will be randomized to either treatment with SPP-001 or placebo. Patients in both treatment groups will be followed until death.

Increased and/or decreased dose(s) and/or administration schedule(s) involving single or multiple dosing of various time intervals can be used.

Evaluation

Measurable lesions (from one to three lesions) should be defined and measured before commencement of SPP-001 treatment. Effect of CR on menopausal symptoms will be registered. Any site effects associated with SPP-001 treatment will be registered. In addi-

tion s-FSH and s-LH will be monitored during the treatment period. Furthermore, quantitative PCR or alike can be applied to determine potential effects on on estrogen regulated genes such as the progesterone receptor gene and the pS2 gene.

At the end of the experiment measurable lesions will be evaluated and any effect according to WHO criterias will be noted.

Survival curves for each group will be constructed and it will be determined whether SPP-001 has any effects on survival in this patient population.

Example 7: CLINICAL EVALUATION OF THE SAFETY OF SPP-001 TREATMENT IN WOMEN WITH ADVANCED BREAST CANCER

This example describes a clinical protocol for safety studies of SPP-001 treatment in women with advanced breast cancer.

Patients

Advanced breast cancer patients who have obtained a complete or partial response upon conventional antineoplastic therapy will be included. The patients should suffer from menopausal symptoms. No chemotherapy or endocrine therapy should be given concomitantly with the SPP-001 treatment. ER status on the cancer cells should be determined.

Treatment

The SPP-001 should be administered orally twice daily at a corresponding dose of 0.40-0.60 mg SPP-001 /kg. Treatment period should be 3 months.

Study design

Patients will be randomized to either treatment with SPP-001 or placebo and followed until death.

Dose(s) and schedule(s) can be subjected to changes.

Evaluation

Time of disease progression using available WHO criterias will be used. Effect of SPP-001 on menopausal symptoms and gonadotropins will be registered. Any side effects associated with SPP-001 composition treatment will be registered.

Time to progression in each treatment group will be determined and compared between groups in order to determine whether SPP-001 has any effect on cancer progression in this patient population.

Example 8: CLINICAL EVALUATION OF THE SAFETY OF SPP-001 IN WOMEN WHO HAVE HAD A PRIOR BREAST CANCER

This example describes a clinical protocol for safety studies of SPP-001 in women with a prior diagnosis of breast cancer and with no indication of recurrence.

Patients

Women with a prior diagnosis of breast cancer who are suffering from menopausal symptoms will be included. The patients must have no sign of recurrent disease. No anti-neoplastic treatment should be given concomitantly with the SPP-001 treatment. ER status on the cancer cells should be determined.

Treatment

The SPP-001 should be administered orally twice daily at a corresponding dose of 0.40-0.60 mg SPP-001 /kg. Treatment period should be 1 year.

Study design

Patients should be randomized to either treatment with SPP-001 or placebo. Patients in both groups will be followed for 5 years.

Dose(s) and schedule(s) can be subjected to changes.

Evaluation

Time to recurrence of disease will be determined. Effect of SPP-001 on menopausal symptoms and gonadotropins will be registered. Any side-effects associated SPP-001 treatment will be registered.

Univariate recurrence-free survival and overall survival curves will be constructed for both treatment groups and compared in order to establish whether the treatment has any effect on these two parameters.

Example 9: EFFECT OF SPP-001 ON ESTROGEN DEFICIENCY CONDITIONS OTHER THAN MENOPAUSAL SYMPTOMS IN PATIENTS WITH CURRENT BREAST CANCER, WITH A PRIOR DIAGNOSIS OF BREAST CANCER, OR WITH AN INCREASED RISK OF DEVELOPING BREAST CANCER

This example describes SPP-001 treatment of estrogen deficiency conditions including dermatological disorders such as ageing of the skin, dryness of mucous membranes (e.g. vaginal and intestine), brain related disease such as Alzheimer's including other types of dementia, bone and joint related disease such as osteoporosis, osteochondrosis, osteo-arthritis, rheumatoid arthritis, healing of bone fractures, and reduce in skeletal fractures and disease such as hyperlipidaemia, hypercholesterolaemia, arteriosclerosis, *etc.* in the subgroup of women who are currently suffering from breast cancer, who have a prior history of breast cancer, or women with an increased risk of developing breast cancer.

Patients

Women belonging to one of the above mentioned breast cancer related groups and suffering from estrogen deficiency, will be candidates for the treatment. Diseases related to estrogen deficiency includes among others those mentioned above.

In addition, women belonging to one of the above mentioned three breast cancer related groups and suffering from diseases which can be cured or relieved by estrogen replacement therapy, can be included in this treatment study.

Treatment

SPP-001 should be administered twice daily at a corresponding dose of 0.40-0.60 SPP-001 /kg.

Dose(s) and schedule(s) can be subjected to changes.

Example 10: CLINICAL EVALUATION OF PHYSIOLOGICAL ESTROGENIC EFFECT OF SPP-001 IN MENOPAUSAL WOMEN

This example describes methods to determine physiological estrogenic effects of SPP-001 and similar compositions in the human female suffering from estrogen deficiencies.

Patients

Women with high FSH and/or elevated LH as a reaction to estrogen deficiency

Material and design

Serum levels of FSH and LH will be determined pre-treatment and at 1 month and 2 months of treatment. Standard commercial assays will be used. Vaginal smear cytology will be performed before and after treatment for 60 days. Bone mineral density will be determined pre-treatment and following two months of treatment. Standard DXA assay will be used.

Example 11: SKIN APPLICATIONS OF SPP-001 OR ALIKE FOR CONDITIONS WHICH COULD BE ALLEVIATED OR CORRECTED BY ESTROGENS

Material

Humans with as for examples wrinkles, dry skin and other estrogen deficiency related conditions.

Treatment

Individuals with the conditions in question are treated with daily SPP-001 or similar with relieve of conditions as endpoint. Typical treatment period is 1-2 months. Route of administration is preferably direct skin application but can also be oral or parenteral.

Example 12: VAGINAL APPLICATION OF SPP-001 TO ALLEVIATE SYMPTOMS OF VAGINAL DRYNESS AND ESTROGEN DEFICIENCY RELATED CONDITIONS

Materials

Women with symptoms of vaginal estrogen deficiency such as dryness and dyspareuni.

Treatment

An appropriate dose of SPP-001 are given to alleviate the vaginal symptoms by direct application to the mucous membrane or by oral or parenteral administration. Used when needed.

Example 13: APPLICATIONS OF SPP-001 OR ALIKE FOR CORONARY HEART APPLICATION (CHD) CONDITIONS WHICH COULD BE ALLEVIATED OR CORRECTED BY ESTROGENS

Estrogens have been generally regarded as cardiac protective in the female population. SPP-001 and alike can be used because of their estrogen beneficial activity, but without is unwanted negative issues, for treatment and alleviation of symptoms of arterial and venous diseases, including heart and brain.

Material

Humans with as for examples arteriosclerosis.

Treatment

Individuals with the conditions in question are treated with daily SPP-001 or similar with a lowering of cholesterol or death in cardiac disease as endpoint. Typical treatment period is from 2 months to 5 years months. Route of administration is preferably orally, but can also be oral or parenteral.

REFERENCES

1 Beuscher, N. Cimicifuga racemosa L. - Die Traubensilberkerze. Z.Phytotherapei, 16: 301-310, 1995.

2 Brünner, N., Bastert, G.B.A., Poulsen, H.S., Spang-Thomsen, M., Engelholm, S.A., Vindeløv, L., Nielsen, A., Tommerup, N., and Elling, F. Characterization of the T61 human breast carcinoma established in nude mice. Eur.J.Cancer Clin.Oncol., 21: 833-843, 1985.

3 Brünner, N., Bronzert, D., Vindeløv, L.L., Rygaard, K., Spang-Thomsen, M., and Lippman, M.E. Effect on growth and cell cycle kinetics of estradiol and tamoxifen on MCF-7 human breast cancer cells grown in vitro and in Nude mice. Cancer Res., 49: 1515-1520, 1989.

4 Brünner, N., Moser, C., Clarke, R., and Cullen, K. IGF-I and IGF-II expression in human breast cancer xenografts: relationship to hormone independence. Breast Cancer Res.Treat., 22: 39-45, 1992.

5 Brünner, N., Osborne, C.K., and Spang-Thomsen, M. Endocrine therapy of human breast cancer grown in nude mice. Breast Cancer Res.Treat., 10: 229-242, 1987.

6 Brünner, N., Spang-Thomsen, M., Poulsen, H.S., Engelholm, S.A., Nielsen, A., and Vindeløv, L. Endocrine sensitivity of the receptor-positive T61 human breast carcinoma serially grown in nude mice. Int.J.Cancer, 35: 59-64, 1985.

7 Brünner, N., Spang-Thomsen, M., Poulsen, H.S., Vindeløv, L.L., and Engelholm, S.A. Characterization of the T60 human breast carcinoma grown in nude mice. In: J. Rygaard, N. Brünner, N. Græm and M. Spang-Thomsen (eds.), Immune-Deficient Animals in Biomedical Research, pp. 234-241, Basel: Karger. 1987.

8 Brünner, N., Spang-Thomsen, M., Vindeløv, L., and Nielsen, A. Effect of 17□-oestradiol on growth curves and flow cytometric DNA distribution of two human breast carcinomas grown in nude mice. Br.J.Cancer, 47: 641-647, 1983.

9 Brünner, N., Spang-Thomsen, M., Vindeløv, L., Nielsen, A., Engelholm, S.A., and Svenstrup, B. Dose-dependent effect of 17 β-estradiol determined by growth curves and flow cytometric DNA analysis of a human breast carcinoma (T61) grown in nude mice. Exp.Cell Biol., 53: 220-232, 1985.

10 Brünner, N., Spang-Thomsen, M., Vindeløv, L., Wolff, J., and Engelholm, S.A. Effect of tamoxifen on the receptor-positive T61 and the receptor-negative T60 human breast carcinomas grown in nude mice. Eur.J.Cancer Clin.Oncol., 21: 1349-1354, 1985.

11 Brünner, N., Svenstrup, B., Spang-Thomsen, M., Bennet, P., Nielsen, A., and Nielsen, J. Serum steroid levels in intact and endocrine ablated BALB/c nude mice and their intact littermates. J.Steroid Biochem., 25: 429-432, 1986.

12 Düker, E.-M., Kopanski, L., Jarry, H., and Wuttke, W. Effects of extracts from Cimicifuga racemosa on godnadotropin release in menopausal women. Planta Med., 57: 420-424, 1991.

13 Harnischfeger, G. and Stolze, H. Traubensilberkerze. Notabene Medici, 10: 446-450, 1980.

14 Jarry, H., Gorkow, C., and Wuttke, W. Treatment of menopausal symptoms with extracts of Cimicifuga Racemosa: In vivo and in vitro evidence for estrogenic activity. Unknown, 1999.

15 Liske, E. and Düker, E. Cimicifuga racemosa in Klinik und Forschung. Ars Medici, 7: 426-430, 1993.

16 Martin, P.M., Horwitz, K.B., Ryan, D.S., and McGuire, W.L. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. Endocrinology, 103: 1860-1867, 1978.

17 McGuire, W.L. Current status of estrogen receptors in human breast cancer. Cancer, 36: 638-644, 1975.

18 McGuire, W.L. Steroid receptors in human breast cancer. Cancer Res., 38: 4289-4291, 1978.

19 Rygaard, K. and Spang-Thomsen, M. Quantitation and Gompertzian analysis of tumour growth. Breast Cancer Res.Treat., 46: 303-312, 1997.

20 Warnecke, G. Beeinflussung klimakterischer Beschwerden durch ein Phytotherapeutikum. Medwelt., 36: 871-874, 1985.

21 Vorberg, G. Therapie klimakterischer Beschwerden. Z.Allg.Med., 60: 626-629, 1984.

22 Gottardis MM., Wagner RJ., Borden EC., and Jordan VC. Differ ntial ability of antiestrogens to stimulate breast cancer cell (MCF-7) growth in vivo and in vitro. Cancer Res 1989 49(17):4765-69

Claims

1. Use of a substance, capable of inducing a physiological estrogen-like effect without interacting with breast cancer cells, in particular without stimulating breast cancer cells, for the preparation of a medicament for the treatment of estrogen deficiency symptoms or diseases of a mammal suffering from or having a high risk of developing breast cancer.

2. Use according to claim 1, wherein the substance is capable of inducing a physiological estrogen-like effect without stimulating breast cancer cells.

3. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is uterine growth as determined by an increase in uterine weight compared to controls after administration of the substance to ovariectomized female athymic nude mice.

4. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is uterine growth as determined by an increase in mean uterine weight compared to controls of at least 0.10 g after administration of the substance to ovariectomized NMRI female athymic nude mice for 8 days.

5. Use according to any of claims 3 or 4, wherein the increase in uterine weight obtained by administration of a dose comparable to a normal dose for the mammal to be treated of the substance corresponds to a weight increase obtainable in the same test animal by estradiol treatment.

6. Use according to any of claims 3 or 4, wherein the increase in uterine weight obtained by administration of a dose comparable to a normal dose for the mammal to be treated of the substance corresponds to a substantially maximum weight increase obtainable in the same test animal by estrogen treatment.

7. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is a change in gonadotropins (FSH and/or LH) as determined by available validated radioimmuno assay techniques.

8. Use according to any of the preceding claims, wherein the physiological estrogen-like effect is a change in cytology of the vaginal cells as determined by cytological counts.

9. Use according to any of the preceding claims, wherein the substance do not interact, in particular stimulate, cancer cells that are estrogen receptor-negative.

10. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the substance compared to a control on growth of the estrogen and progesterone receptor negative MDA-MB-231 (ATCC HTB-26) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

11. Use according to any of the preceding claims, wherein the substance do not interact, in particular stimulate, cancer cells that are estrogen receptor-positive.

12. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the substance compared to a control on growth of the estrogen dependent and estrogen receptor-positive MCF-7 (ATCC HTB-22) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

13. Use according to any of the preceding claims, wherein the lack of stimulation of breast cancer cells is determined by no effect of the substance when given in combination with estradiol compared to a control on growth of the estrogen dependent and estrogen receptor-positive MCF-7 (ATCC HTB-22) human breast cancer cell line inoculated into six-week-old female athymic nude mice determined when control tumours show increasing size during at least six consecutive growth recordings.

14. Use according to any of the preceding claims for the treatment of estrogen deficiency symptoms or diseases of humans having breast cancer, having a high risk of recurrent breast cancer, or having a risk (such as high risk) of developing breast cancer.

15. Use according to any of the preceding claims, wherein the estrogen deficiency-conditioned symptom or disease is selected from the group consisting of menopausal symptoms; dermatological disorders such as ageing of the skin, wrinkles, dry skin and other

estrogen deficiency related dermatological disorders; dryness of mucous membranes (e.g. vaginal and intestine); brain related disease such as Alzheimer's including other types of dementia; bone and joint related diseases such as osteoporosis, osteochondrosis, osteoarthritis, rheumatoid arthritis, healing of bone fractures, and reduction in skeletal fractures; vaginal estrogen deficiency such as vaginal dryness and dyspareuni; coronary heart diseases such as arteriosclerosis; and disease such as hyperlipidaemia and hypercholesterolaemia.

16. Use according to any of the preceding claims, wherein the estrogen deficiency-conditions symptoms are menopausal symptoms.

17. Use according to any of the preceding claims, wherein the composition is a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

18. Use according to any of the preceding claims, wherein the composition is or contains *Cimicifuga Racemosa* extract.

19. Use according to any of the preceding claims, wherein the composition is a composition comprising *Cimicifuga Racemosa* plant parts.

20. Use according to any of the preceding claims, wherein the composition is a composition comprising SPP-001.

21. Use according to any of the preceding claims, wherein the composition is a composition containing one or more chemical compounds contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

22. Use according to any of the preceding claims, wherein the composition is combined with a drug which has a selective estrogen receptor modulating (SERM) actitivty.

23. A container comprising a substance according to any of the preceding claims with a pharmaceutically carrier and comprising an indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening estrogen dependent cancer.

24. A container comprising a substance capable of inducing a physiological estrogen-like effect without stimulating breast cancer cells with a pharmaceutically carrier and comprising an indication for relief of estrogen deficiency symptoms without increasing the risk of developing or worsening estrogen dependent cancer.

25. A container according to any of the previous claims, wherein the substance is extracted from *Cimicifuga Racemosa*.

26. A method for relieving symptoms caused by estrogen deficiency in a mammal suffering from or having a high risk of developing an estrogen dependent tumour comprising administering to the mammal a substance, capable of inducing a physiological estrogen-like effect without stimulating breast cancer cells.

27. A method according to the previous claim, wherein the mammal is a human.

28. A method for screening for substances or compositions which can be used according to claim 1 or 2, comprising subjecting test substances or compositions to

3) testing for possible estrogen-like effect in normal tissue by measuring increase in uterine weight, changes in gonadotropins, changes in vaginal cytology and/or postmenopausal symptoms in an adult female mammal and
4) testing for possible estrogenic effect in breast cancer, and selecting, as candidates for tissue-selective estrogenic substances or compositions useful in the method according to claim 1 or 2, substances or compositions which,
   c) are capable of inducing physiological estrogenic effects in female mammals, and at the same time
   d) have no effect on the growth of estrogen receptor-negative cancer cells and no effect on estrogen receptor-positive cancer cells in the doses in which they induce physiological estrogen effects.

29. A method according to claim 28, wherein the capability of the substance or composition of inducing physiological estrogen effects e.g. uterine growth female mammals is tested by testing the capability of the substance or composition of effecting uterine weight increase in ovariectomized female NMRI athymic nude mice, the lack of effect of the substance or composition on the growth of estrogen receptor-negative cancer cells is as-

sessed as the lack of capability of the substance or composition of supporting growth of MDA-MB231 xenografts in female NMRI athymic nude mice, and the lack of effect of the substance or composition on the growth of estrogen receptor-positive cancer cells is assessed as the lack of capability of the substance or composition of supporting growth of MCF-7 (ATCC (HTB-22) xenografts in female NMRI athymic nude mice.

30. A method for relieving or curing symptoms or diseases which are caused by estrogen deficiency, or which can be relieved or cured by administration of steroidal estrogen, in a mammal who suffers from breast cancer, or has a risk of recurrent breast cancer, or has a high risk of developing breast cancer,
the method comprising administering, to the mammal, a composition
which has an estrogen-like effect, as evidenced by a capability of the composition of inducing physiological estrogenic effects in adult mammal, and
which is free from interaction with breast cancer cells, in particular free from a stimulating effect on breast cancer,
thereby treating estrogen deficiency symptoms or diseases without introducing a risk of provoking the development of clinically evident breast cancer and/or stimulating growth of existing breast cancer cells in the mammal.

31. A method according to claim 30, wherein the mammal is female mammal.

32. A method according to claim 31, wherein the female mammal is a woman.

33. A method according to any of claims 30-32, wherein the estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing an increase in uterine weight in adult ovariectomized NMRI female athymic nude mice.

34. A method according to claim 33, wherein the increase in uterine weight following a dose comparable to a normal dose for the mammal to be treated corresponds to a weight increase seen in the same test animal following estradiol treatment.

35. A method according to claim 34, wherein the increase in uterine weight following a dose comparable to a normal dose for the mammal to be treated corresponds to a substantially maximum weight increase obtainable in the same test animal by estrogen treatment.

36. A method according to any of claims 30-35, wherein the estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing a lowering in FSH and LH in females.

37. A method according to any of claims 32-36, wherein the estrogen-like effect possessed by the composition manifests itself in the composition being capable of inducing an estrogen like change in vaginal cytology in females.

38. A method according to any of claims 30-37, wherein the composition is one which has no effect on the growth of estrogen receptor-negative cancer cells.

39. A method according to claim 38, wherein the composition is one which has no effect on the growth of xenografts of the estrogen and progesteron receptor-negative MDA-MB-231 (ATCC HTB-26) human breast cancer cell line in nude mice.

40. A method according to any of claims 30-39, wherein the composition is one which is free from any effect on breast cancer cells even where the breast cancer cells are documented as being estrogen receptor-positive.

41. A method according to any of claims 30-40, wherein the composition is one which has substantially no agonizing and substantially no antagonizing effect on the effect of estrogen such as estradiol on breast cancer cells, even where the breast cancer cells are documented as being estrogen receptor-positive.

42. A method according to claim 41, wherein the composition is one which substantially does not bind to estrogen receptors of cancer cells.

43. A method according to any of claims 40-42, wherein the composition is one which has no effect on xenografts of the estrogen receptor-positive and estrogen dependent MCF-7 (ATCC HTB-22) human breast cancer cell line in nude mice, as evidenced by the composition having no growth supportive effect and no growth inhibitory effect on the xenografts whether given alone or in combination with estradiol.

44. A method according to claim 43, wherein the composition is one which has no effect on xenografts of the estrogen receptor-positive and estrogen dependent MCF-7 (ATCC HTB-22) human breast cancer cell line in nude mice, as evidenced by the composition having no growth supportive effect and no growth inhibitory effect on the xenografts whether given alone or in combination with estradiol, even where the composition is administered in a dose which is 10 or even 100 times higher than a dose giving, in the same strain of nude mice, a maximum uterus weight increase.

45. A method according to any of claims 30-44, wherein the estrogen deficiency-conditioned symptom or disease is selected from the group consisting of menopausal symptoms, dermatological disorders such as ageing of the skin, dryness of mucous membranes (e.g. vaginal and intestine), brain related disease such as Alzheimer's including other types of dementia, bone and joint related disease such as osteoporosis, osteochondrosis, osteoarthritis, rheumatoid arthritis, healing of bone fractures, and reduce in skeletal fractures and disease such as hyperlipidaemia, hypercholesterolaemia, arteriosclerosis.

46. A method according to claim 45, wherein the estrogen deficiency-conditions symptoms are menopausal symptoms.

47. A method according to any of claims 30-46, wherein the composition is a composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

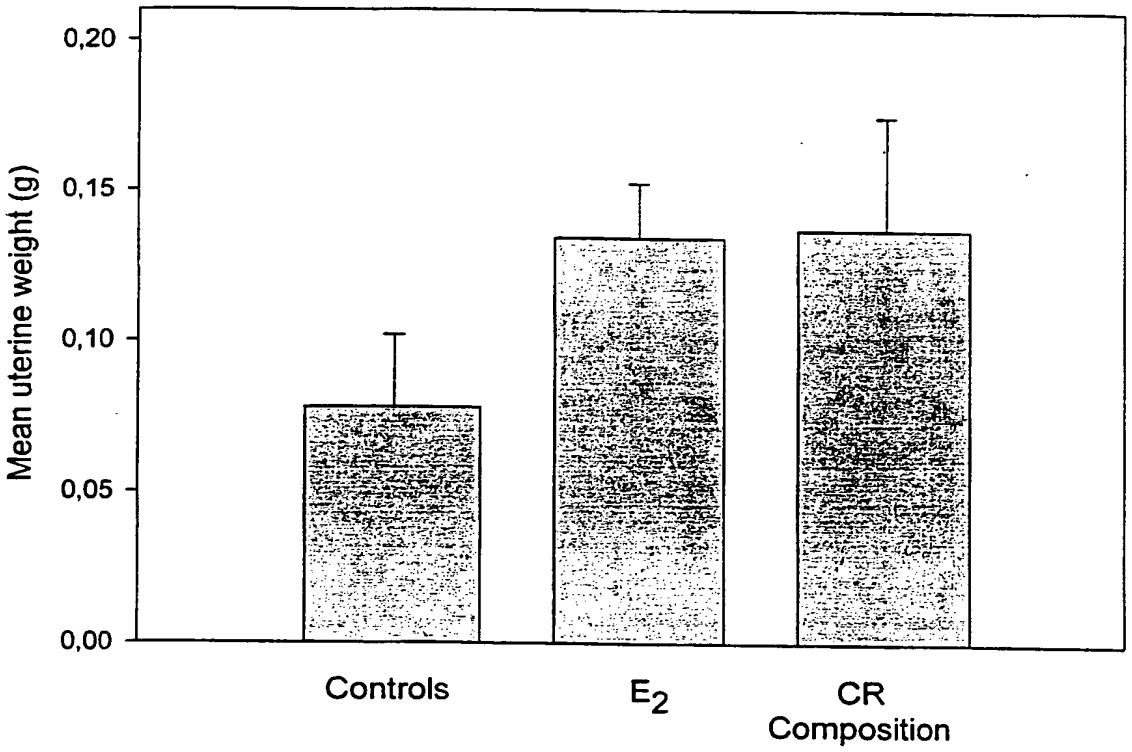
48. A method according to claim 47, wherein the composition is or contains *Cimicifuga Racemosa* extract.

49. A method according to claim 47, wherein the composition is a composition comprising *Cimicifuga Racemosa* plant parts.

50. A method according to claim 47, wherein the composition is a composition comprising SPP-001.

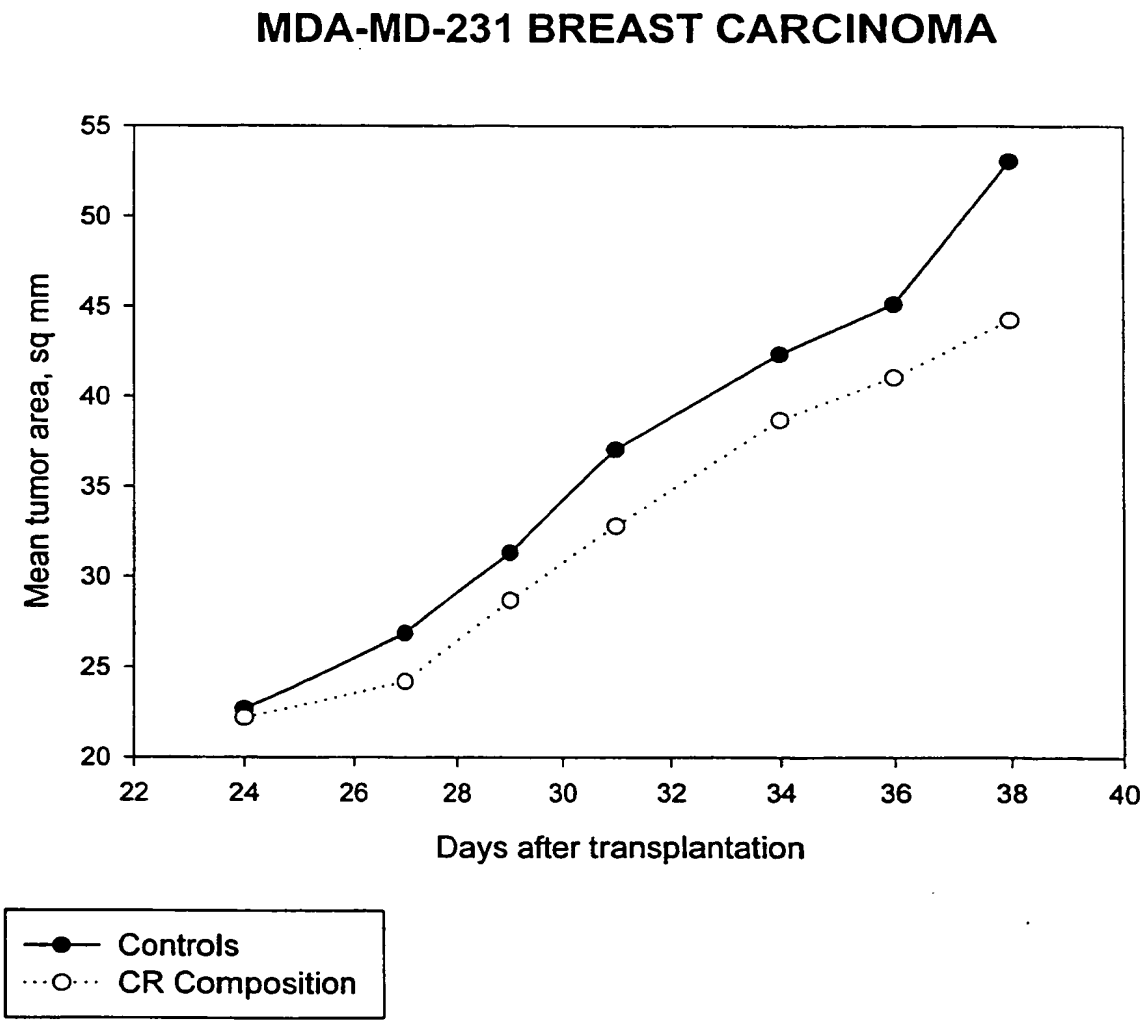
51. A method according to claim 48, wherein the composition is a composition containing one or more chemical compounds contained in *Cimicifuga Racemosa* extract, or derivatives thereof.

52. A method according to claim 30, wherein the composition is combined with a drug which has a selective estrogen receptor modulating (SERM) activity.



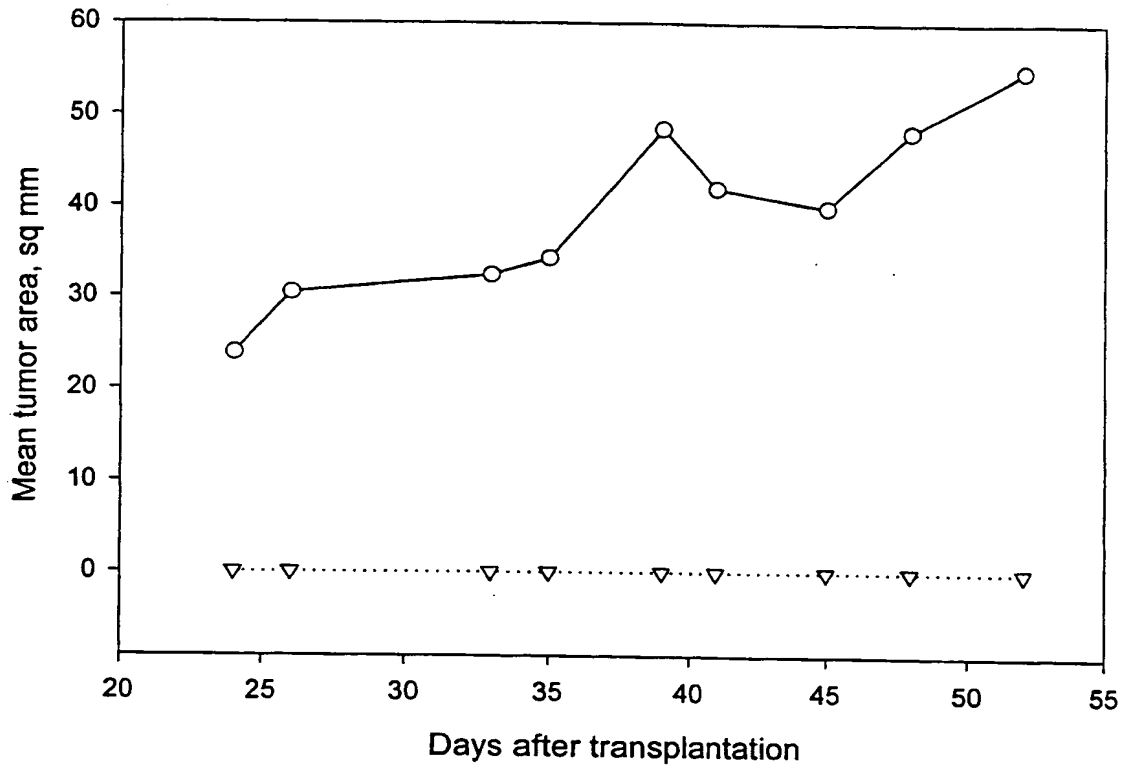
Effect of estradiol (E_2) and CR Composition on mouse uterine weight.

Fig. 1



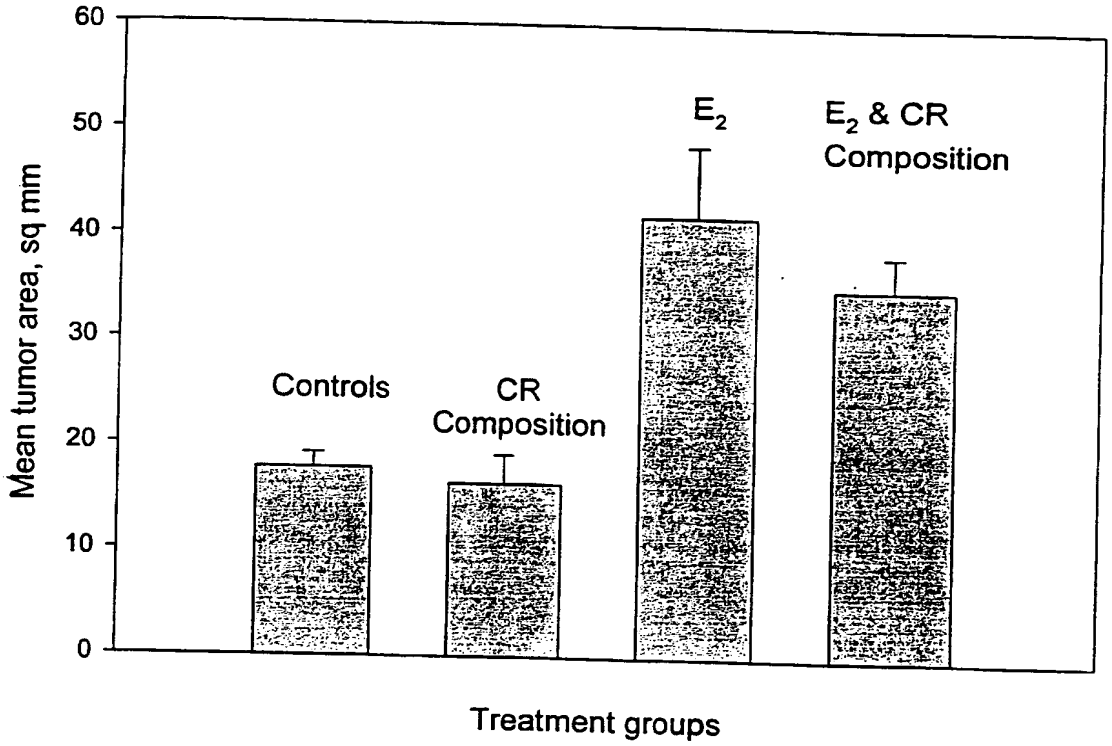
Mean tumor area growth curves of human MDA-MB-231 breast carcinoma xenografts. The mice were untreated controls or treated with CR Composition.

Fig. 2



Mean tumor area growth curves of human MCF-7 breast carcinoma xenografts. The mice were treated with E_2 or CR Composition. The lack of tumor growth in CR Composition treated mice are indicated in the figure.

Fig. 3



Mean tumor size of MCF-7 xenografts in nude mice calculated from tumor measurements obtained at day 20 after transplantation. The calculations were Based on 10, 5, 8, and 11 measurable tumors in the Control, CR Composition, E2, and combined treatment groups, respectively.

Fig. 4

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 January 2001 (25.01.2001)

PCT

(10) International Publication Number
WO 01/05415 A3

(51) International Patent Classification[7]: A61K 35/78, A61P 15/12

(21) International Application Number: PCT/DK00/00406

(22) International Filing Date: 17 July 2000 (17.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 1999 01034 15 July 1999 (15.07.1999) DK

(71) Applicant *(for all designated States except US)*: **KØBENHAVNS UNIVERSITET** [DK/DK]; Nørregade 10, DK-1070 København K (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BRÜNNER, Nils** [DK/DK]; Brodersens Allé 1, DK-2900 Hellerup (DK). **SPANG-THOMSEN, Mogens** [DK/DK]; Lille Bygade 15, DK-2635 Ishøj (DK).

(74) Agent: **PLOUGMANN, VINGTOFT & PARTNERS A/S**; Sankt Annæ Plads 11, DK-1250 København K (DK).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
25 May 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/05415 A3

(54) Title: COMPOSITION HAVING STEROIDAL ESTROGEN EFFECT WITHOUT INCREASING THE RISK OF BREAST CANCER

(57) Abstract: The present application relates to a pharmaceutical composition containing substances contained in *Cimicifuga Racemosa* extract, or derivatives thereof with steroidal estrogen effect. This composition, however, does not have the steroidal strogen side-effect of increasing the risk of development or the tendency to relapse of breast cancer and are thus suitable for relieving or curing symptoms or diseases which are caused by estrogen deficiency or which can be relieved or cured by administration of steriodal estrogen, in women, who suffer from breast cancer, or have a risk of recurrent breast cancer, or have a high risk of developing breast cancer. The application further relates to the uses of such compositions and methods for identifying same.

US

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
21497 PC 1		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/DK 00/ 00406	17/07/2000	15/07/1999
Applicant		
KØBENHAVNS UNIVERSITET et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT
Information on patent family members

02/11/00

International application No.
PCT/DK 00/00406

EP	0847755	A1	17/06/98	AU	4830097	A	18/06/98
				AU	5549098	A	15/07/98
				CA	2245890	A	25/06/98
				DE	19652183	C	12/02/98
				HU	9901360	A	30/08/99
				IL	125776	D	00/00/00
				NO	975858	A	15/06/98
				PL	323617	A	22/06/98
				WO	9826791	A	25/06/98

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00406

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 35/78, A61P 15/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Fortschr. Med., Volume 114, No 5, 1996, Von G. Rauthe, "Wechseljahresbeschwerden bei Mammakarzinompatientinnen", page 26; page 29 - page 30, see page 47/29 --	1-52
A	Journal of women's health. Mary Ann Liebert, Inc., Volume 7, No 5, 1998, Shari Lieberman, Ph.D., C.N.S., "A Review of the Effectiveness of Cimicifuga racemosa (Black Cohosh) for the Symptoms of Menopause" page 525 - page 529 --	1-52
A	EP 0847755 A1 (SCHAPER & BRüMMER GMBH & CO. KG), 17 June 1998 (17.06.98) -- --------	1-52

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 November 2000	01 02 2001

Name and mailing address of the International Searching Authority
European Patent Office P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel(+31-70)340-2040, Tx 31 651 epo nl,
Fax(+31-70)340-3016

Authorized officer
Carolina Gómez Lagerlöf/EÖ
Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK00/00406

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 26-52
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☒ Claims Nos.: 1-16,22-24
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Box I.1

Claims 26-52 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

Box I.2

Present claims 1-16 and 22-24 relate to a substance defined by reference to a desirable characteristic or property, namely capable of inducing a physiological estrogen-like effect without interacting with breast cancer cells. The claims cover substances having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and / or disclosure within the meaning of Article 5 PCT for only a very limited number of such substances. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lacks clarity (Article 6 PCT). An attempt is made to define the substance by reference to a result to be achived. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to substances that are extracted from *Cimicifuga Racemosa*.